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HOSPITAL LABORATORY METHODS

FOR

STUDENTS, TECHNICIANS AND CLINICIANS

M C JUNKIN

Hospital Laboratory Methods

FOR

Students Technicians and Clinicians

BY

FRANK A. McJUNKIN, A. M., M. D.

PROFESSOR OF PATHOLOGY, MARQUETTE UNIVERSITY SCHOOL OF MEDICINE
MILWAUKEE, WIS.

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PREFACE

The purpose of this book is to place in the hands of the hospital laboratory worker and the technician of other small laboratories, directions for the tests that are commonly required on specimens sent to such laboratories.

These specimens may be grouped as clinical laboratory (urine, gastric contents, feces, sputum, and blood), bacteriological, surgical and autopsy specimens. The manner in which the material should be handled before and after it reaches the laboratory is indicated in a specific way. There are usually several methods for obtaining the same results but in no case has an attempt been made to enumerate these; the simplest procedure by which the object of the analysis can be obtained with the greatest certainty has been chosen. No methods that are new and not thoroughly tried out have been given.

The interpretation of facts ascertained by chemical and cytological examinations is very often possible only after consideration of the clinical condition of the patient. To place interpretations on laboratory findings, therefore, necessitates a thorough consideration of the various diseases and this has not been attempted.

In our hands, the methods described below are used by the medical students taking clinical pathology and those electing a course in pathologic technic and by non-medical technicians to whom a course covering twenty-four weeks is offered during three consecutive summers.

For whatever merit there is in these methods, I wish to acknowledge my indebtedness to Dr. F. B. Mallory of the Harvard Medical School under whom I received instruction while student and assistant in the laboratory of the Boston City Hospital.

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HOSPITAL LABORATORY METHODS

URINE

Introduction.—Each hospital patient has a 3-liter, narrow-mouthed bottle provided with a funnel and containing 10 c.c. of toluol. Chloroform has been widely used for preserving urine for chemical examination but it cannot be entirely driven off by heat. As soon as the urine is voided it is emptied into this bottle and the total amount passed during the twenty-four-hour period is collected. This twenty-four-hour sample is used for chemical analysis. The sample for microscopic examination is sent to the laboratory in a pint clamp-stoppered tonic or beer bottle within an hour after it is voided. If the sample for microscopic purposes cannot be sent to the laboratory soon after it is voided a small amount of 10 per cent. formalin may be added. Urine to which formalin has been added is not suitable for chemical analysis.

Normal urine when voided is usually clear; rarely in strongly alkaline urine there is a cloudiness due to precipitated phosphates and carbonates. Soon a cloud (nubecula) is formed by the collection of epithelial cells on the mucin present. The color varies from colorless to dark amber. The amount is from 800 c.c. to 3000 c.c. An alkaline reaction in the fresh urine is due to alkaline (disodium) phosphates and to carbonates, while the acid reaction usually present results from an excess of dihydrogen phosphates. There may be such a combination of these phosphates that the reaction is amphoteric. The specific gravity is taken with a urinometer (Fig. 1). It has a normal variation between 1.010 and 1.025.

Albumin.—The usual abnormal protein substances present are serum albumin and serum globulin but nucleoalbumin, mucin and albumose may be present. Resins, uric acid, urates, urea and the bile acids may with some tests react like albumin. If the urine is

not clear, filter it before testing for albumin or add kieselguhr and filter.

Heller's Test (Fig. 2).—Place 5 c.c. concentrated nitric acid (*a*) in a test-tube and stratify the urine (*c*) above the acid by slanting the test-tube and allowing the clear urine to trickle down its side

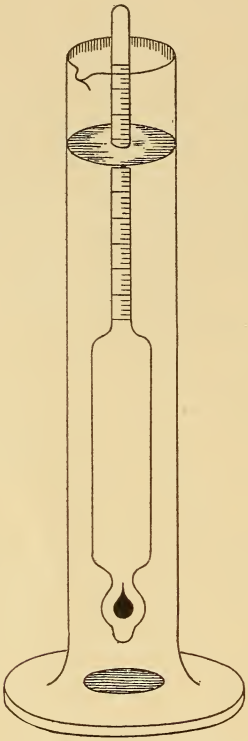


Fig. 1.—Urinometer.

Fig. 2.

Fig. 2.—Urine. Heller's Test. *a*, Nitric acid; *b*, precipitate at surface of contact; *c*, filtered urine; *d*, filter paper; *e*, 5-cm. funnel. The test-tube is heavy glass with a non-flaring mouth 155 by 16 mm. outside measurements. A medium-size tube for Wassermann work, etc., measures 120 by 13 mm. A small serum tube measures 55 by 7 mm. These test-tubes are obtained from Bausch & Lomb Optical Co.

from the funnel (*e*) while filtering. A white ring (*b*) at the surface of contact may be due to albumin. When the Heller test is positive, protein substances other than albumin must be ruled out by further observations and tests. Nucleoalbumin and mucin produce

a ring several millimeters above the surface of contact and on gentle agitation this clouding ascends. If due to albumose, on applying gentle heat the ring disappears. Therefore, to rule out nucleoalbumin, mucin and albumose in all cases in which the ring is obtained, agitate and then apply gentle heat.

As regards non-protein substances, if resins are suspected add a few drops of concentrated hydrochloric acid to 10 c.c. of the original urine and shake. Resins are precipitated. The remaining substances, uric acid, urates, urea and the bile acids, do not give the test unless the urine is concentrated. Of these substances urates most commonly give a precipitate. A precipitate due to urates disappears on heating.

Heat and Acetic Acid Test.—If a ring is obtained with Heller's test, the heat and acetic acid test should be applied. To 20 c.c. urine in a test-tube, add 5 drops 20 per cent. acetic acid and 5 c.c. of a saturated solution of sodium chloride. Boil the upper half of the contents of the tube, using the lower half as a control. The sodium chloride keeps the nucleoalbumin and the mucin in solution, while albumose is soluble in the heated solution. Uric acid, urates, urea and the bile acids do not precipitate. If a precipitate of resins is suspected, cool and shake with 10 c.c. of benzol. Any precipitate of resins goes into solution in the benzol.

Albumose (and Bence-Jones body) form a coagulum at about 60°C. in the heat and acetic acid test but the precipitate almost entirely disappears when the boiling point is reached.

Rough Estimation of Albumin by Esbach Method.—Fill the albuminometer (Esbach obtained from Spencer Lens Co., Buffalo, N. Y.) to the mark "U" with acidified urine and to the mark "R" with Esbach's reagent (picric acid 1, citric acid 2, and distilled water 100). Stopper, thoroughly mix by inverting and set aside in a test-tube rack for twenty-four hours. The reading on the tube that corresponds to the upper surface of the precipitate is the number of grams per liter of protein. Less than 0.5 gm. per liter will not sediment. This determination of protein is not accurate and has a doubtful value.

If an accurate quantitative estimation of albumin is desired, the

nitrogen of the proteins may be determined by taking the difference between the total nitrogen and the nitrogen after the precipitation and removal of all proteins present.

Sugar (Glucose).—About $\frac{1}{20}$ per cent. glucose is normally present in the urine. This amount does not react with the usual tests. Levulose gives the same reduction tests as glucose, and when present the polariscopic and reduction quantitative estimates do not check. Maltose has 2.5 times as great dextrorotatory power as glucose and may therefore produce a discrepancy in the polariscopic and other quantitative estimations. Levulose and maltose when present are usually accompanied by glucose and have the same significance. Lactose may be present in the urine of lactating women. Lactose is not fermented by yeasts and reduces copper only slowly. Pentoses are rarely present. They reduce copper slowly and are inactive optically. Glycuronic acid conjugated with other substances such as indoxyl occurs in normal and pathologic urines. With Fehling's test they give a greenish reduction. If there is a suspicion of glucose, make the phenylhydrazin test. With this test no crystals are obtained with the glycuronates. Homogentisic acid which is present in the urine of alkaptonurics reduces Fehling's solution, but is readily recognized owing to the urine turning brown and then black on standing exposed to the air.

Fehling's Test.—If the urine is not clear, add lead acetate and filter. Remove albumin by the heat and acetic acid method. Place 5 c.c. Fehling's solution *A* (copper sulphate, Merck reagent, 34.65 gm., distilled water 1000 c.c.) and 5 c.c. Fehling's solution *B* (Rochelle salt 173 gm., sodium hydrate 125 gm., and distilled water 1000 c.c.) in a test-tube and boil. Remove from flame and at once add about 1 c.c. urine and allow a few minutes for reduction to take place. A reduction is shown by the appearance of a brick red precipitate.

Nylander's Test.—To prepare Nylander's reagent dissolve 4 gm. Rochelle salt in 100 c.c. of warm 10 per cent. sodium hydrate, add 2 gm. bismuth subnitrate, shake, and filter into a dark-colored bottle. To 10 c.c. urine from which all albumin has been removed, add 1 c.c. of the reagent and place in boiling water

in a water-bath for five minutes. If positive, the urine darkens and the black precipitate settles out during the heating. Uric acid, creatinin, and the alkaptone bodies may produce a dirty

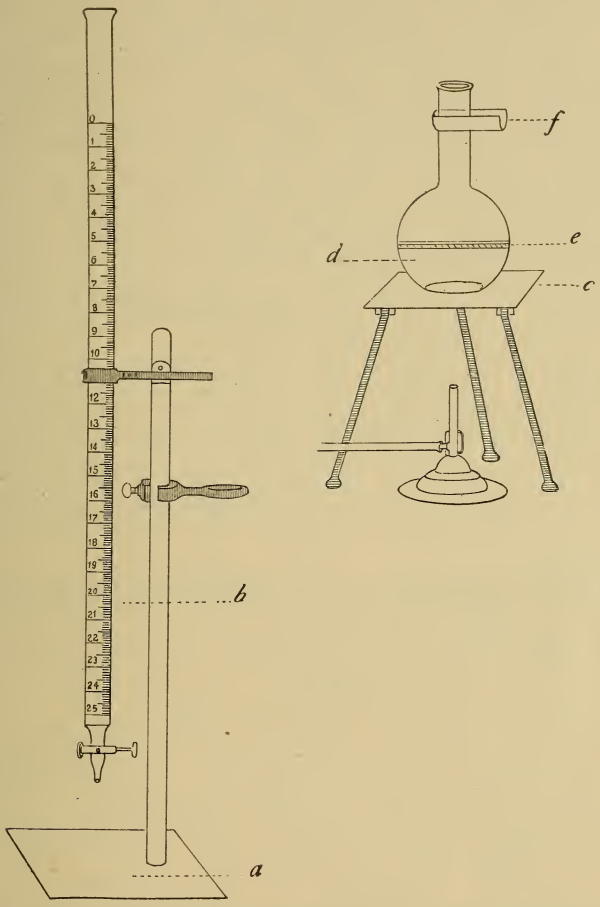


FIG. 3.—URINE. Quantitative Fehling's Test. *a*, White base or base covered with filter paper; *b*, burette containing the diluted urine; *c*, asbestos gauze; *d*, solution; examine for the clear zone, *e*, against a white background after removing from the flame; *f*, folded muslin to be held with thumb and finger.

yellow color (atypical reaction) with Fehling's test but do not react to Nylander's test. Chloroform must be removed before applying tests for sugar. Urine to which formalin has been added must never be used.

Phenylhydrazin Test.—In cases in which the reduction is not altogether typical this test should be applied. To 5 c.c. urine in a test-tube add 5 drops of a pure phenylhydrazin base (Merck & Co.) and 10 drops of glacial acetic acid. Boil one minute. Add 5 drops 15 per cent. sodium hydroxide and heat again for a few seconds and then set aside to cool. The yellow sheaf-like crystals appear at once or within twenty minutes. The crystals obtained from the bottom of the tube with a pipette are examined under the high dry objective. If the specific gravity of the urine is high the sample should be diluted until it is below 1.020.

Polariscopic Determination of Glucose.—Two grams of lead acetate are added to the twenty-four-hour sample of urine. After filtering, fill the 189.4 mm. glass tube with the clear urine, apply the glass disk, being careful not to get an air bubble beneath it, and screw on the metal cap. Place the tube in the polariscope and starting at zero turn the handle until the field is equally illuminated. The percentage of sugar is read off from the vernier provided the instrument is made for sugar estimation only (saccharometer).

Quantitative Determination of Glucose by Fehling's Method.—Place 10 c.c. (accurate) Fehling's *A* (10 c.c. of Fehling's *A* contains 0.3465 gm. copper sulphate) and 10 c.c. of Fehling's *B* (approximate) in a 300-c.c. Florence flask of Jena glass (Fig. 3) and dilute with about 50 c.c. of water. Heat to boiling and run in less than 1 c.c. of the diluted (If the specific gravity of the urine is 1.030 or over dilute it 1–10 with water; if under 1.030 dilute 1–5 with water.) urine from a burette (*b*) and again boil. Repeat this until no blue color remains. Examine for this end point (absence of bluish tinge) by looking against a white sheet of paper on the clear line (*e*) 3 mm. below the top of the meniscus. A yellowish change in the red precipitate indicates that too much urine has been added: 0.05 gm. of glucose are required for the reduction of this amount of Fehling's. For example if 8 c.c. of a 1–5 dilution is required to discharge the blue, 8 c.c. of this dilution or 1.6 c.c. of the undiluted urine contains 0.05 gm. glucose; 1 c.c. of the undiluted urine 0.032 gm.; 100 c.c. contains 3.2 gm. or 3.2 per cent.

A *simple method for computing dilutions* should be used. By a dilution one in ten (1–10) is meant such a dilution that in 10 parts of it there is 1 part of the original liquid. To make such a dilution take 1 part of the original liquid and 9 parts of water. More complicated dilutions are often required; for example, from a 14 per cent. solution (14–100), make a 3 per cent. solution (3–100). One part of the 14 per cent. solution contains $^{14}/_{100}$ part of the original liquid, while 1 part of the required 3 per cent. solution contains $^{3}/_{100}$ part of the original solution. Therefore the 14 per cent. solution is $^{14}/_{100}$ divided by $^{3}/_{100}$ or 4.66 times too strong. Therefore take 1 *part* of the 14 per cent. solution and the *remainder* of water which is 3.66 parts.

Quantitative Determination of Glucose by Fermentation.—Dilute the urine so that the specific gravity is below 1.008 and the percentage of sugar below 1. Fill with the diluted urine the test-tube that comes with the Einhorn saccharometer (obtained from Spencer Lens Co., Buffalo, N. Y.) to the mark and add a knife-point of fresh compressed yeast. Shake well, fill the saccharometer displacing all air in the closed end and place in the incubator for twenty-four hours. The percentage of sugar is indicated on the blind arm by the amount of gas present. Multiply this by the dilution made to get the percentage of sugar.

This method gives only an approximate result and should not be used in careful work for quantitative determinations.

Acetone (Lange).—To 20 c.c. urine add 1 c.c. of a 10 per cent. solution of sodium nitroprusside in 10 per cent. acetic acid. Mix, and then stratify a few cubic centimeters of strong ammonia on the surface. If a violet color appears at the surface of contact and persists, it is most likely due to acetone. To stratify any liquid slant the tube (Fig. 2) and carefully let the lighter liquid flow down the side of the inclined tube.

Acetone (Gunning).—Place 100 c.c. of urine in an extra large test-tube or in a small Erlenmeyer flask and add 1 c.c. concentrated hydrochloric acid. Stopper with a rubber cork carrying a glass tube 2 feet long curved at an angle of 45 degrees. Boil and collect the first 10 c.c. of distillate in a test-tube. To 5 c.c. of this add

5 drops of ammonia and iodine solution (iodine 4 gm., potassium iodide 6 gm. and water 100 c.c.) until the black precipitate formed does not immediately dissolve. If acetone is present a yellow precipitate of iodoform soon forms. Examine this precipitate for hexagonal crystals using the high dry lens. A positive test is produced only by acetone.

Diacetic Acid.—To 10 c.c. urine, add 10 drops 10 per cent. ferric chloride solution and filter. The excess of phosphates is removed by the filtration. Now add a few drops more of the ferric chloride solution. If diacetic is present a red color appears. Place 10 c.c. urine in a second test-tube, boil for several minutes and proceed with the test exactly as above. If the red color was due to diacetic acid, it should be much less intense or entirely absent in the boiled urine.

Oxybutyric Acid (Black).—Evaporate 10 c.c. urine in a small porcelain dish to about one-third, acidify with a few drops of hydrochloric acid and add with a spatula plaster of Paris to a thick paste. When it begins to set break up into a meal with the spatula, add 30 c.c. ether and mix. Decant the clear ether (20 c.c.) into a dry evaporating dish, evaporate the ether over a water-bath and dissolve the residue in water (10 c.c.). Neutralize with an excess of dry barium carbonate (about 12 gm.), pour into a test-tube, add a few drops of hydrogen peroxide and 5 drops of 10 per cent. ferric chloride. A red color indicates oxybutyric acid.

Bile Pigment (Gmelin).—Stratify the urine above concentrated nitric acid. If bile pigment is present a greenish color appears at the point of contact.

To confirm this test for bile pigment, dilute tincture of iodine with 95 per cent. alcohol until it is straw-colored and then stratify the iodine solution on the urine. A greenish ring at the contact indicates bile pigment (Smith).

Diazo-reaction (Ehrlich).—To 10 c.c. sulphanilic acid solution (sulphalic acid 5, hydrochloric acid 50, and water 1000), add 0.5 c.c of 0.5 per cent. sodium nitrite solution and 10 c.c. urine. Mix and then stratify with concentrated ammonia. If positive

a pink ring forms at the surface of contact and when the mixture is shaken the foam appears pink.

Indican (Jaffé).—To 2 c.c. chloroform in a test-tube add 5 c.c. urine, 5 c.c. concentrated hydrochloric acid, and 2 drops of a strong calcium hypochlorite suspension(10 gm. in 100 c.c. water). Shake. A blue color in the chloroform is positive. Iodides may give a confusing red to purplish color. If they are suspected add a few drops of 10 per cent. sodium hyposulphite solution and shake. This removes any color in the chloroform due to iodides.

Chlorides (Volhard).—To 5 c.c. urine (albumin-free) in a small Erlenmeyer flask add 20 c.c. distilled water, 10 c.c. silver

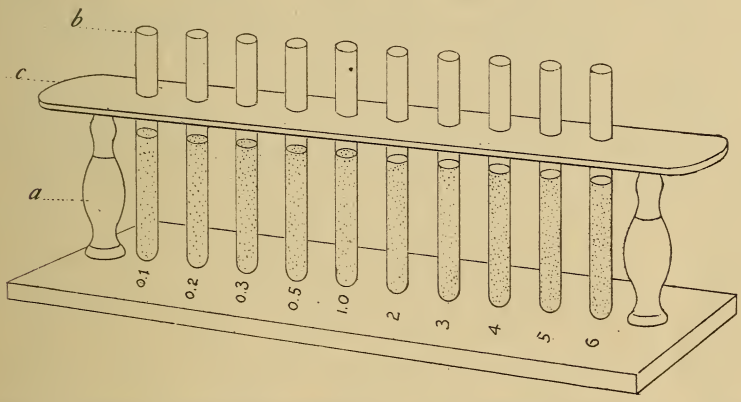


FIG. 4.—URINE. Tubes for phthalein test. *a*, Narrow wooden rack containing ten test-tubes *b*, 155 by 16 mm. These tubes contain standard solutions of phenolphthalein. Stopper with small corks. The top part of the rack *c* should be within one inch of the top of the tubes.

nitrate solution (29.042 gm. silver nitrate in 1 liter of water), and 2 c.c. indicator (30 c.c. water + 70 c.c. nitric acid, specific gravity 1.2 + excess of ferric ammonium sulphate: filter). Now run in ammonium sulphocyanate (3.25 gm. ammonium sulphocyanate + 250 c.c. distilled water − 1 c.c. of this solution must be equivalent to 1 c.c. of the silver solution) until the first red appears and persists when stirred. 1 c.c. of the silver solution is equivalent to 0.01 gm. sodium chloride.

Phthalein Test (Rowntree and Geraghty) (Fig. 4).—Have the patient drink a pint or more of water and catheterize the

bladder. Then inject intramuscularly 1 c.c. of a solution containing 6 mg. of phenolsulphophthalein. To 0.6 gm. phenolsulphophthalein add 1 c.c. of N-sodium hydrate and 100 c.c. saline. Of this solution give 1 c.c. Hynson, Wescott & Co., Charles St., Baltimore, place the preparation on the market in 1 c.c. ampules. The catheter is left in place and the urine allowed to flow into a container in which a drop of strong sodium

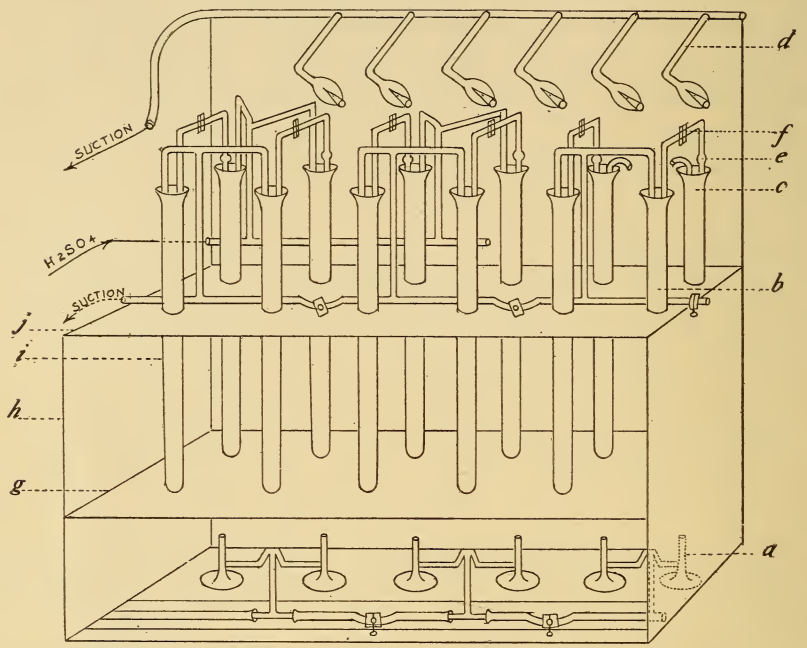


FIG. 5.—URINE. Folin apparatus for nitrogen determinations. *a*, Three pairs microburners under three pairs digestion tubes, *c*, that are provided with fume absorbers, *d*; *b*, three pairs of tubes into which ammonia is aspirated; *e*, pipette for alkali; *f*, pinch cock in rubber connections; *g*, wire gauze covered with asbestos in which are holes for bottom of tubes; *h*, *i* and *j*, are boards.

hydrate has been placed until the first red color appears (ten minutes normally) when the catheter is removed. At the end of the first hour the patient urinates into a clean container, and at the end of a second hour into a second container. Place the first- and second-hour specimens in two separate 1000 c.c. cylinders and add distilled water to 1000 c.c. Make each alkaline. Compare these

with standard samples in ten tubes (Fig. 4) containing 0.1, 0.2, 0.3, 0.5, 1.0, 2, 3, 4, 5, 6 mg. per 1000 c.c.

Total Nitrogen in Urine (Folin and Farmer) (Fig. 5).—Place 5 c.c. urine in a 50-c.c. graduated flask and dilute up to volume. To 1 c.c. of diluted urine measured into a 25 × 200-mm. test-tube (Jena) (*c*) provided with a fume absorber (Fig. 6) add 1 c.c. sulphuric acid by means of a piette, 1 gm. potassium sulphate, 1 drop 10 per cent. copper sulphate and a glass bead. Boil over a micro-burner for five to ten minutes to complete digestion with the fume absorber (*d*) in place. Cool until viscous, detach the fume absorber, and slowly add 6 c.c. of water. Add 3 c.c. of 40 per cent. NaOH by drawing it into the pipette (*e*) containing 1 drop caprylic alcohol and holding it there by means of a pinch cock (*f*) until the corks are inserted for aspiration into the front tubes (*b*). The suction is now started slowly at first and then continued more rapidly for fifteen minutes in order to aspirate all the ammonia into the front tubes (*b*) which contains 10 c.c. one-fiftieth normal HCl, 3 drops of a 1 per cent. alizarine red solution and 30 c.c. water. At the end of fifteen minutes wash the delivery tube and titrate the excess of acid in the tubes (*b*) with one-fiftieth normal NaOH (end reaction is a red color). One cubic centimeter of one-fiftieth normal acid neutralized by the aspirated ammonia is equivalent to 0.00028 gm. nitrogen. Specimens are always examined in duplicate. It is convenient to have a rack with six tubes in the rear for the digestion and six in front for the ammonia absoprtion. With such an apparatus three separate determinations can be made at the same time.

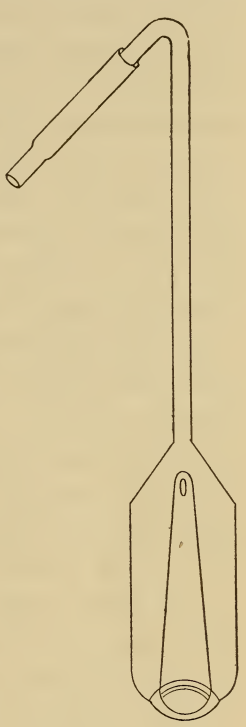


FIG. 6.—URINE. Folin fume absorber. Made by Eimer and Amend or it may be blown from suitable glass tubing.

Urea Nitrogen.—Measure into tube (*b*), Fig. 5, 25 c.c. of one-

fiftieth normal hydrochloric acid, 3 drops of alizarin red and 1 drop of caprylic alcohol. In tube (*c*) place 5 c.c. of urine diluted 1–10, 1 c.c. of 15 per cent. urease in water (the urease is obtained from Arlington Chemical Co., Yonkers, N. Y.) and 1 drop of caprylic alcohol. Stopper (*c*) and (*b*) and allow to stand fifteen minutes then pass air for one-half minute to remove any ammonia fumes in the upper part of the tube. Add 5 gm. potassium carbonate to (*c*) and pass air for fifteen minutes. Each cubic centimeter of one-fiftieth normal acid neutralized is equivalent to 0.00028 gm. nitrogen. Find the number of grams of nitrogen that would be liberated from 100 c.c. of urine by this test. From this amount subtract the number of grams of ammonia nitrogen in 100 c.c. of the urine and the remainder will be the number of grams of urea nitrogen in 100 c.c. of the urine.

Rough Determination of Urea by Ureometer.—Add to the small side tube of the ureometer (Doremus-Hinds obtained from Spencer Lens Co., Buffalo, N. Y.) 1 c.c. urine allowing it to flow in through and fill the opening in the glass cock. Close the cock and wash out with water any urine that has run into the bulb portion of the instrument, after which it is ready for the addition of the hypobromite solution.

To prepare the hypobromite solution place in a large test-tube 13 c.c. Rice's solution *A* (bromine 10 c.c., potassium bromide 31 gm., distilled water 250 c.c.) and 15 c.c. of Rice's solution *B* (sodium hydrate 100 gm., and distilled water 250 c.c.). Pour this into the bulb portion of the apparatus incline so as to displace the air from the blind arm, completely filling it with the hypobromite mixture. Fill the small side tube with urine and allow exactly 1 c.c. of urine to flow slowly into the bulb portion. Each large division of the blind arm represents 1 per cent. urea. The large divisions are divided into tenths. For example, if the column of gas collecting in the blind arm measures 0.014, then the percentage of urea would be 1.4.

The determination of urea by this method when most carefully carried out is not an accurate one and it has a doubtful use.

Ammonia Nitrogen (Folin-Macallum).—Place 2 c.c. urine in the rear tube and add 0.5 c.c. ammonia reagent (15 gm. potassium carbonate, 15 gm. potassium oxalate, water 100 c.c.) and 2 drops of caprylic alcohol. Connect up at once and proceed as in the total nitrogen determination.

Uric Acid (Folin and Shaffer).—In each of two beakers place 150 c.c. fresh urine and add to each 37.5 c.c. Folin-Shaffer reagent (500 gm. ammonium sulphate, 5 gm. uranium acetate, 60 c.c. of 10 per cent. acetic acid and 500 c.c. of distilled water). Stir vigorously and filter after a few minutes. Measure 125 c.c. of the filtrate (100 c.c. of urine) into a beaker and add exactly 5 c.c. concentrated ammonia, cover the beaker and allow to stand forty-eight hours. At the end of this time filter off the ammonium urate and wash several times with 10 per cent. ammonium sulphate. Now wash the precipitate back into the beaker with exactly 100 c.c. of distilled water and 20 c.c. of concentrated sulphuric acid and titrate immediately with one-twentieth normal potassium permanganate (1.5780 gm. per liter) until the first permanent pink color appears. Each cubic centimeter of permanganate equals 3.76 mg. of uric acid. The two samples must check.

Sediment.—To obtain sediment for microscopic examination, freshly voided urine is centrifuged. The sediment is taken from the bottom of the centrifuge tube with a small pipette, transferred to a slide and examined with the low power. If necessary drop a cover on the sediment and examine with the high dry. A reagent may be drawn under one side of the cover by applying filter paper to the opposite side. In examining unstained objects cut the light down to a minimum. The Abbé condenser may be swung out to advantage.

Surgical Urines.—The urine should be examined before a general anesthetic is administered. In hospitals where there are very large surgical wards these examinations may become burdensome. In such cases rather than carelessly examine the urine the above technic should be altered somewhat. Urine cylinders with a cone at the bottom (obtained from Arthur Thomas and Co.,

Philadelphia) are placed on a table and all the urine emptied into these before beginning the tests. Remove the slip from the bottle and place it under the cylinder at the time the urine is poured into it. To test for albumin use a modified Heller technic by substituting a piece of glass tubing a foot long with a 3-mm. lumen for the test-tube. Place the tubing in the urine in the cylinder to a depth of 1 inch and then retain the urine by firmly pressing the thumb over the top. Lower into a graduate containing concentrated nitric acid until 1 inch of the acid is forced into the tube. Now remove the pipette from the acid with the thumb firmly on the top and examine for a white ring at the contact. On lowering the tube into the nitric do not remove the thumb from the top until it is lowered to the level of the urine in the pipette.

To test for sugar, add a few drops from each of about five urines and perform the Fehling's test as usual. If positive then go back and determine which urine gave the positive test.

To obtain sediment from the bottom of the cone, lower the glass tubing to the bottom holding the thumb over the top and then allow the desired amount of sediment to flow into it. Place a drop of the sediment on a glass slide large enough for at least five sediments. This method of examination should be used only when a more careful examination is prohibitive.

The Unorganized Sediment of Acid Urine (Fig. 7).—In acid urine, amorphous urates (quadriurates of sodium and potassium) separate out on cooling and absorb urochrome (yellow) and uroerythrin (red) from the urine. They dissolve on heating. Hydrochloric acid dissolves amorphous urates and later uric acid crystals form. Uric acid, usually as whetstones, may separate out from the urine without the addition of acid. The uric acid crystals are insoluble in hydrochloric acid and soluble in mineral acids and insoluble in acetic. Calcium oxalate usually forms octahedra that are soluble in mineral acids. Cholesterin forms rhombic plates that turn carmine and then violet on the addition of sulphuric acid. Hematoidin in icteric urine may form needles or rhombs. They are colored but do not react like uric acid. Cystin

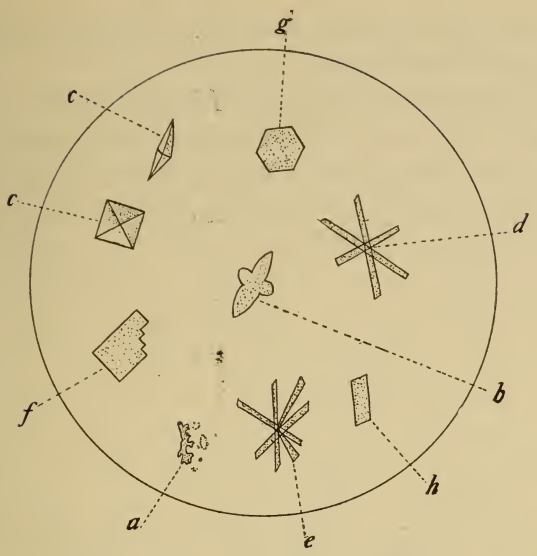


FIG. 7.—URINE. Unorganized sediment of acid urine. *a*, Amorphous quadriurates of potassium and sodium; *b*, uric acid; *c*, calcium oxalate; *d*, monocalcium phosphate; *e*, calcium sulphate; *f*, cholesterin; *g*, cystin; *h*, hematoidin.

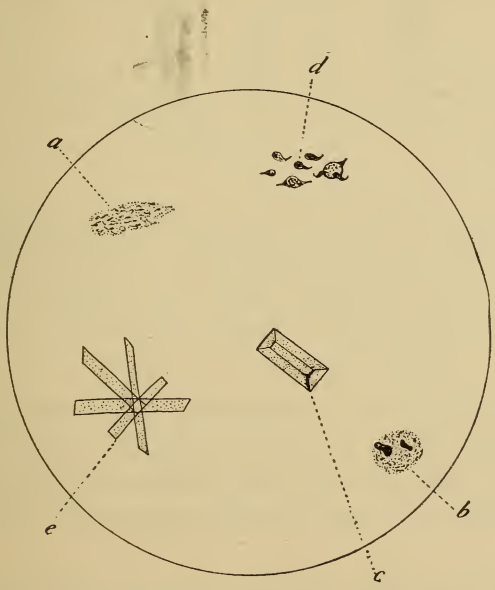


FIG. 8.—URINE. Unorganized sediment of alkaline urine. *a*, Tricalcium and trimagnesium amorphous phosphates; *b*, amorphous calcium carbonate; *c*, ammonium-magnesium phosphate; *d*, ammonium biurate; *e*, dicalcium or dimagnesium phosphate.

occurs as colorless hexagonal plates that readily dissolve in hydrochloric acid and in ammonia.

The Unorganized Sediment of Alkaline Urine (Fig. 8).—In alkaline urine tricalcium and trimagnesium phosphates (amorphous phosphates) form white deposits soluble in acids. Calcium carbonate is like these except that the addition of acid to it causes the evolution of gas.

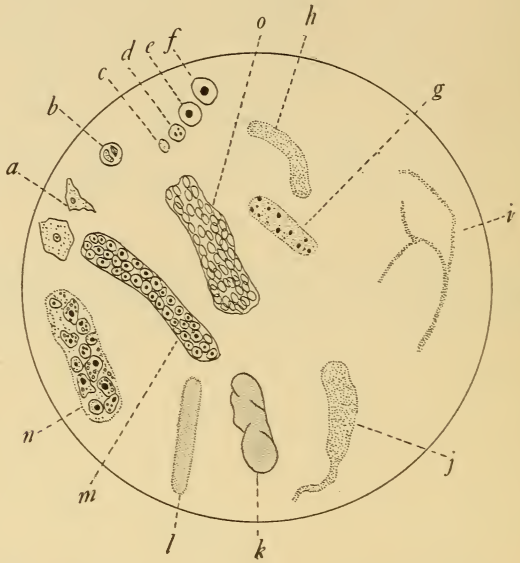


FIG. 9.—URINE. Organized sediment. *a*, Squamous epithelium from ureter, bladder, urethra, prepuce, vulva or vagina; *b*, endothelial leucocyte with blood pigment; *c*, red blood corpuscle; *d*, neutrophile (pus cell); *e*, round epithelial cell from the small ducts of the genital apparatus; *g*, coarse granular cast; *h*, fine granular cast; *i*, mucous thread; *j*, cylindroid; *k*, waxy cast; *m*, pus cast; *n*, epithelial cast; *o*, blood cast. Examine with high dry under cover-glass after preliminary examination with low power without cover.

Ammonium magnesium phosphate (triple phosphate) is the common "coffin lid" crystal of alkaline urine. Ammonium biurate forms yellowish spheres or "morning star" crystals. Rarely dicalcium and dimagnesium phosphates form prisms in the urine.

Organized Sediment (Fig. 9).—Tissue cells and débris are spoken of as organized sediment in distinction to unorganized amorphous and crystalline sediment.

Epithelial Cells.—It is not at present possible to name the source of all epithelial cells from their morphologic appearance. Numerous small round cells when occurring with albumin may, perhaps, be considered as coming from the tubules of the kidney. It must be remembered that small round cells also come from small ducts of the male genitals. The ureter, bladder, urethra, prepuce, vulva, and vagina desquamate squamous epithelium.

Pus.—Neutrophiles (pus cells) have finely granular cytoplasm with polymorphous nuclei that are well brought out by allowing 3 per cent. acetic to flow under the cover. The presence of casts may indicate that the pus comes from the kidney. The source of the pus is best cleared up by cystoscopic examination and ureteral catheterization. Contamination of the specimen with leucorrheal discharge must be eliminated. Endothelial leucocytes containing blood pigment may be found in the urine.

Blood.—Red blood corpuscles appear under the high dry lens as typical disks or only as shadows. Menstrual blood must be excluded. A test for chemical blood (guaiac) (see p. 24) is often desirable.

Casts.—They are formed by the collection of foreign substances in the tubules of the kidney. Epithelial casts are made up largely of dead desquamated epithelium from the tubules. Pus casts are formed by the fusion of neutrophiles collected in the tubules. Blood casts are clots of blood formed in the tubules. Hyaline casts are formed by the discharge of hyaline droplets from the kidney cells. Hyaline casts are homogeneous. Waxy casts are more refractive than the hyaline casts, show fissures and frequently give the amyloid reaction with iodine. Epithelial, pus, and blood casts may form coarsely and finely granular casts when degeneration occurs and the cells disintegrate. *Cylindroids* are hyaline in appearance but one end tapers off into a thread. They are said to have the same significance as hyaline casts.

Mucous threads form the nubecula and are often present as twisted bands. In distinction to casts they are insoluble in acetic acid.

"Clap" threads are made up of heavy mucous material that has collected many pus or epithelial cells. They are macroscopic.

GASTRIC JUICE

Introduction.—Vomitus may serve for the demonstration of blood but its examination is otherwise of little value. Suitable material for chemical and microscopic examination is obtained by passing the stomach tube. This is done in the morning before any food or drink has been taken (fasting stomach), one hour after a test breakfast (bread-water breakfast), or four hours after a test dinner (meat dinner).

Fasting Stomach.—If more than 10 c.c. is obtained when the stomach tube is passed in the morning there is retention and gross and microscopic food particles should be looked for. Titrate the acidity and examine for lactic acid according to the directions given under chemical examination after a bread-water breakfast.

Bread-water Breakfast.—Give one shredded wheat biscuit and 1 pint of water or weak tea without sugar or cream and introduce the stomach tube at the end of one hour. With normal motility there is obtained less than 100 c.c. of odorless, colorless fluid containing particles of the carbohydrate food given.

Bile gives a green or yellow color. Blood may be fresh, but more often is partially digested giving the "coffee-ground" appearance. Lactic, butyric and other organic acids give a sour odor. A putrid odor may result from necrotic stomach wall or from protein decomposition of retained food. An excess of mucus is shown by the tenaciousness of the material when the contents are poured onto the filter.

Microscopic Examination (Fig. 10).—Filter into a 100-c.c. graduate using a 10-cm. funnel. Make a microscopic examination of the sediment on the filter. Starch granules show concentric laminations. Fat globules are highly refractive. Blood corpuscles are not commonly seen as they rapidly disappear in the stomach contents. The nuclei of pus cells may persist after the cytoplasm has disappeared. Budding yeasts and yeasts in large

numbers are not normally present. Sarcines are abnormal and are most often found in cases of benign stasis. Oppler-Boas bacilli form lactic acid and are most often found in cases of malignant disease.

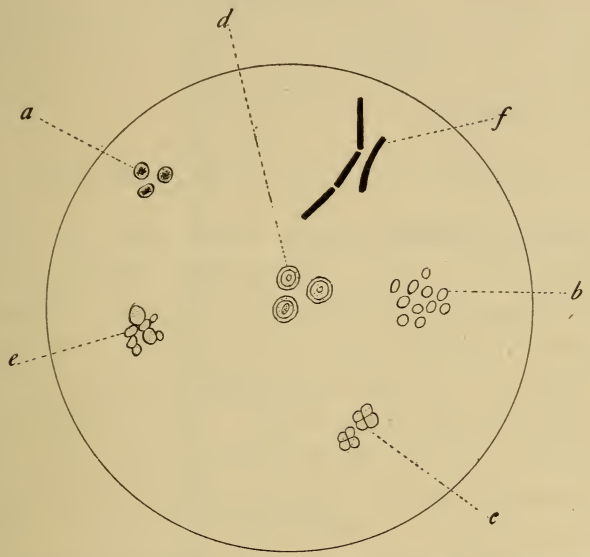


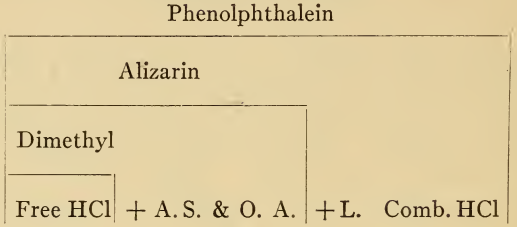
FIG. 10.—GASTRIC CONTENTS. Microscopic findings, examine with high dry. *a*, Fat globules; *b*, red blood corpuscles; *c*, sarcines; *d*, starch granules; *e*, yeasts; *f*, Oppler-Boas bacilli.

Chemical Examination.—One gram of the precipitate on the filter and 1 c.c. of the filtrate are placed together in a test-tube and examined for occult blood by the guaiac test (p. 24); the other chemical tests are performed on the filtrate.

Günzberg's Test for Free Hydrochloric Acid.—This acid is normally present in excess (so-called free hydrochloric acid) and the determination of its presence is important. Günzberg's test is made by adding 5 drops of the filtrate to 5 drops of Günzberg's reagent (phloroglucin 2 gm., vanillin 1 gm., and absolute alcohol 30 c.c. The reagent must not be older than two months) in a small porcelain dish which is evaporated to dryness on a water bath. A bright red color shows the presence of a mineral acid.

As an additional test Congo paper may be placed in the filtrate.

If free hydrochloric acid is present the paper is turned blue. Günzberg's reagent reacts to a smaller amount of a mineral acid than is shown by this test.



Quantitative Titration of the Acids (Fig. 11).—Place 5 c.c. gastric filtrate in a 30-c.c. Erlenmeyer flask (*e*), add 1 drop dimethylamidoazobenzol (½ per cent. alcoholic solution) and run in decinormal NaOH until the bright red color is lost and a yellowish color appears. If there is no free HCl, no red color is developed when the indicator is added. If for example, 1 c.c. tenth-normal alkali is required to discharge the red color, then 1 times 20 or 20 c.c. decinormal solution would be required to neutralize 100 c.c. of the filtrate using the dimethyl as an indicator. The amount of decinormal alkali required to neutralize 100 c.c. of the gastric juice is taken as the numerical expression of the acidity. Thus in this case the free hydrochloric is 20. Normally it varies between about 20 and 40. Now add 1 drop phenolphthalein (½ per cent. alcoholic solution) and continue to run in the tenth-normal alkali until the first tinge of pink appears. The number of cubic centimeters of alkali required to produce the pink times 20 gives the total acidity. This should lie between 40 and 70.

To a second 5 c.c. of the filtrate add 1 drop sodium alizarin monosulphonate (½ per cent. aqueous solution) and run in the tenth-normal alkali until the first tinge of red appears. This indicator titrates the organic acids and acid salts in addition to the free HCl. The difference then between the alizarin titration and the dimethyl titration gives the organic acids and acid salts (A. S. & O. A.) (about 4).

The estimation of the organic acids and acid salts by this method is of doubtful value. Lactic acid reacts to the dimethyl

and is the chief source of error. The difference between the total acidity and the alizarin titration gives the loosely combined hydrochloric acid (L. Comb. HCl).

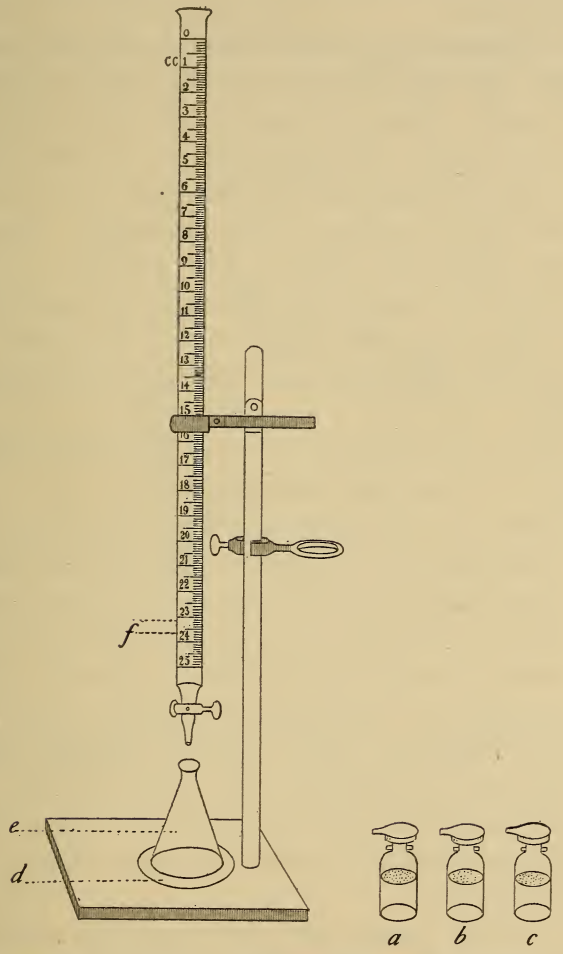


FIG. 11.—GASTRIC CONTENTS. Apparatus for gastric titration. *a*, Dimethyl in 40-c.c. dropping bottle; *b*, alizarin; *c*, phenolphthalein; *d*, filter paper; *e*, 50-c.c. Erlenmeyer flask; *f*, pipette containing decinormal alkali.

Lactic Acid (Kelling).—Place 14 c.c. distilled water in a test-tube and add 1 c.c. gastric filtrate and 1 drop 10 per cent. ferric chloride. To a second tube of exactly the same size add 15 c.c. water and 1 drop 10 per cent. ferric chloride. If lactic acid is

present a canary-yellow color develops in the first tube. This is seen by looking straight into the mouth of the tube against a white background and comparing this tube with the one that contains ferric chloride alone.

Pepsin.—Capillary glass tubes 2 mm. in diameter and 20 cm. long are filled with egg white by suction and the ends plugged with bread crumbs. Boil five minutes. Preserve in glycerine.

Dilute 1 c.c. gastric contents in a 30-c.c. Erlenmeyer flask with 15 c.c. twentieth-normal HCl and place in it 2 cm. of the albumin tube after washing off the glycerine. Place in the incubator for twenty-four hours. The number of millimeters digested from the two ends in this time may be recorded.

Rennin.—Add 5 drops filtrate to 10 c.c. neutral milk and place in the incubator for fifteen minutes. Coagulation shows the presence of rennin.

Starch.—To 5 drops filtrate in a porcelain dish add 5 drops Lugol's iodine. A blue color indicates starch.

Meat Dinner.—At noon give the patient clear broth, ⅓ pound beefsteak, a small amount of mashed potato and one roll.

Introduce the stomach tube in four hours. If the stomach has emptied itself, its motor power is good, and on the following day the stomach tube should be used one hour earlier after administration of the same dinner. Undigested meat at the end of four hours shows deficient digestion. In a normal stomach there may remain on chemical examination only a small amount of almost completely digested food with a trace of free hydrochloric acid. The chemical examination is carried out just as in the case of the bread-water breakfast.

FECES

Introduction.—The fresh specimen should be sent to the laboratory in a covered container and at once place in an incubator until examined. Undigested food particles, substances escaping from the biliary tract, occult blood, and parasites are the usual things examined for.

Undigested Food Particles (Fig. 12).—With a platinum loop place a small amount of the stool on the center of a slide, mix with water if necessary, apply a cover-glass and examine first with the low and then the high objectives.

In faulty digestion muscle fibers are numerous and have distinct striations and square (not rounded) ends. Much fibrous

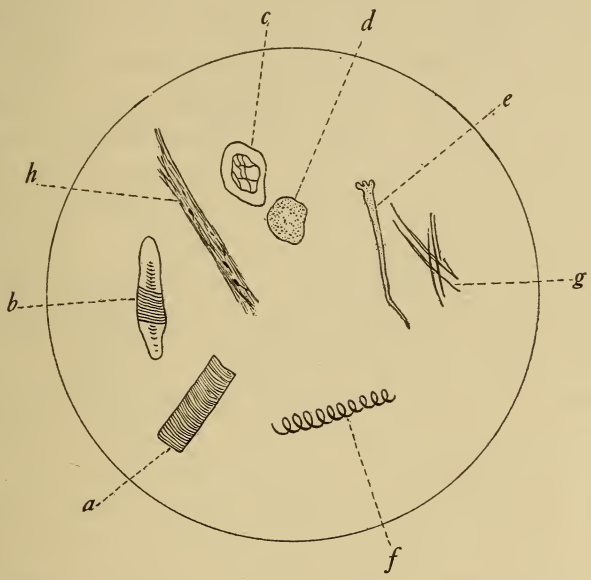


FIG. 12.—FECES. Undigested food particles. *a*, Undigested muscle fiber; *b*, partially digested muscle fiber; *c* and *d*, vegetable cells; *e*, vegetable hair; *f*, vegetable spiral; *g*, fatty acid crystals; *h*, undigested connective tissue.

connective tissue indicates faulty gastric digestion. Vegetable cells, spirals (the vessels of plants), and hairs are numerous in normal stools.

Substances Escaping from the Biliary Tract.—To find gallstones, the stool is tied up in a bag made of two thicknesses of surgical gauze, placed in a vessel and then washed under the tap. After washing out everything possible, cut the string with which the bag is tied, spread out the gauze with forceps and examine for stones.

Bilirubin after escaping into the intestine is reduced by the bacteria present there to hydrobilirubin. To test for this pigment

rub up a gram mass in a mortar with 3 c.c. saturated aqueous solution of bichloride of mercury and set aside twenty-four hours to dry. The particles that contain hydrobilirubin are red while those containing bilirubin are green. (Schmidt).

Occult Blood (Guaiac Test).—Place 1 gm. feces in the bottom of a test-tube with a glass rod, with a platinum loop, or, if fluid, with a pipette and add 5 c.c. glacial acetic acid and shake. Extract with ether by adding 20 c.c. ether to the tube and shaking gently, holding the thumb firmly over the mouth of the tube.

Decant 5 c.c. of the clear supernatant ether extract into a second test-tube, add an equal amount of distilled water, 1 c.c. hydrogen peroxide, a knife-point of powdered guaiac and shake. A blue color in the ether is a positive test. A blue color is given not only by hemoglobin but also by red meats and green vegetables (these should be eliminated from the diet), by pus, by the heavy metals and by certain other things less commonly. A negative test shows the absence of blood.

Parasites.—Examination of stools for parasites is of great importance, especially in the tropics. The specimens for examination should be obtained by giving a saline cathartic.

Bacteria.—The bacterial content of the gastro-intestinal tract has frequently been made the subject of investigation but there are few organisms demonstrable in the intestine as the cause of specific lesions.

The demonstration of *tubercle bacilli* is an indication of tuberculous ulcers of the intestine only when it is known that the patient has not swallowed tuberculous material coughed up from the lungs. The anus must be thoroughly cleansed to eliminate the chance of error due to smegma bacilli present there. Tubercle bacilli are examined for by selecting suspicious looking masses of mucus and staining as in the case of sputum (p. 30).

Dysentery bacilli and bacilli of the *typhoid-colon group* are the commonest bacteria sought for identification. They are identified by isolating the organisms by means of successive streaks on plates of the proper media and agglutination of selected colonies

from the plates with known positive sera specific for the suspected organism.

AMEBA.—*Entameba Histolytica* (Fig. 13).—Choose blood-stained mucus, if present, from the fresh warm stool and place a small particle on a warm slide or warm stage and apply a cover. With the high dry lens this ameba has a clear ectosarc and in its

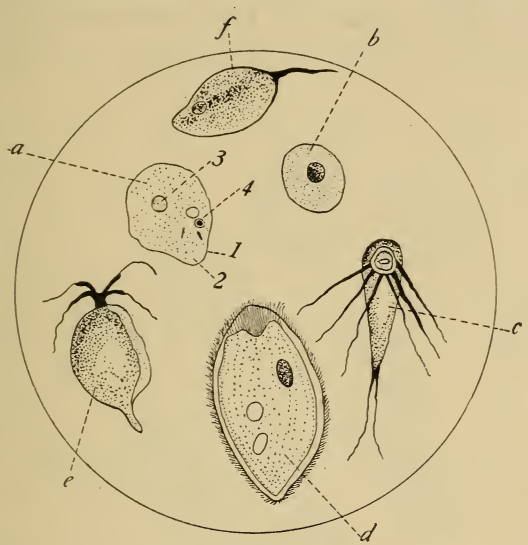


FIG. 13.—FECES. Protozoa. *a*, Entameba histolytica—1, ectoplasm; 2, endoplasm; 3, nucleus; 4, cellular and bacterial inclusions; *b*, entameba coli; *c*, lamblia intestinalis with four pairs of flagella; *e*, trichomonas intestinalis with two pairs of flagella attached to the anterior end; *f*, cercomonas with a single flagellum at its anterior end.

cytoplasm there are usually cells and bacteria. The nucleus is indistinct. This ameba is about five times the size of a red blood corpuscle.

The *entameba coli* is a common inhabitant of the human intestinal tract. It is one to three times the size of a corpuscle, has a distinct nucleus and is only slightly phagocytic for corpuscles and other cells. It is not pathogenic.

FLAGELLATA.—*Cercomonas hominis, trichomonas intestinalis, and lamblia intestinalis* are flagellates found in the intestinal tract.

They may be seen with the high dry objective. They have not been shown to be pathogenic.

INFUSORIA.—*Balantidium coli* is a pathogenic ciliate that produces ulceration of the colon. It may be found in the stools.

NEMATODES.—A large number of the nematodes are parasitic for man. *Uncinaria americana* and *ankylostoma duodenale*, the

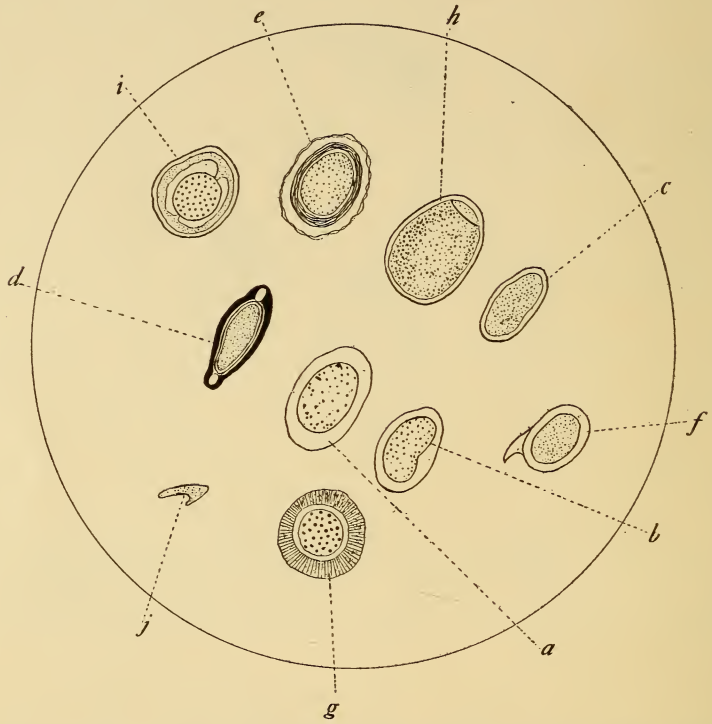


FIG. 14.—FECES. Ova. *a*, Unicaria americana or ankylostoma duodenale; *b*, strongyloides stercoralis; *c*, oxyuris vermicularis; *d*, tricocephalus dispar; *e*, ascaris lumbricoides; *f*, schistosoma hematobium; *g*, tenia saginata or solium; *h*, dibothriocephalus latus; *i*, tenia nana; *j*, hooklet from echinococcus cyst.

new and old world forms of the hook-worm, are the most important of the nematodes (round-worms). The eggs are characteristic and offer the chief means for diagnosis (Fig. 14). Examine by placing a small amount of the liquid stool on the center of a slide, applying a cover and examining with the high dry lens.

For a more searching examination place about one ounce of feces in a pint conical measuring glass, fill up with water and thoroughly mix with a glass rod. After sedimenting pour off the supernatant fluid and repeat. Finally examine the layer of finest sediment.

The adult worm is present in the fresh stool only after treatment. Embryo worms may be obtained by placing ova-containing stools in the incubator for a few days. The ova of the new and old world forms of hook-worm are not readily differentiated.

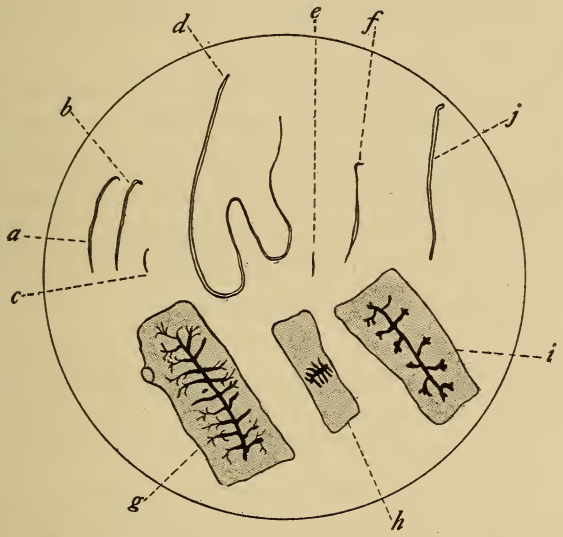


FIG. 15.—FECES. Worms. Natural size. Examine first with low power. *a*, Ankylostoma duodenale; *b*, uncinaria americana; *c*, trichinella spiralis; *d*, tricocephalus dispar; *e*, strongyloides stercoralis; *f*, oxyuris vermicularis; *g*, segment of tenia saginata; *h*, segment of dibothriocephalus; *i*, segment of tenia solium; *j*, tenia nana.

Strongyloides stercoralis is diagnosed by finding the embryos (Fig. 15), as the eggs are not commonly found in the stool. Examine with the high dry lens.

Oxyuris vermicularis is the common pin-worm or seat-worm. Both eggs and adult worms may be found in the feces. The former are most often found in scrapings from about the anus and this is the most important means of diagnosis. Scrape off the material

with a dull scalpel, place on a slide with a very small amount of water and examine with the high dry lens.

Tricocephalus dispar is the whip-worm and is diagnosed by finding the ova in the feces.

Ascaris lumbricoides is the common round-worm. Both the adult worms and the eggs may be found in the stools. The latter may have ragged albuminous capsules or they may be naked.

Trichinella spiralis is difficult to demonstrate in the feces. Place the feces obtained by active purgation in a tall cylinder and dilute with water. Pour the sediment into a large glass dish so as to form a thin layer and examine for minute worms against a black background.

TREMATODES.—Infections with trematodes or fluke-worms are not common in America and the cases found are importations.

Schistosoma Hematobium.—The eggs of this parasite may be found in either the feces or urine. The adult worm lies in the mesenteric vein and in the veins of the bladder and vagina. The eggs penetrate through the walls of these vessels and reach the lumen of the bladder and of the intestine.

CESTODES.—Some of the tapeworms are common intestinal parasites and are often diagnosed by the patient by his finding segments in the stools.

Tenia saginata infections are diagnosed by finding segments by the method used for finding gall-stones or by finding the ova in the stools. The ova from the beef tapeworm will not infect man if ingested as the cysticercus bovis develops only in the beef.

Tenia solium is differentiated from the saginata by finding the segments. The ova of the two are very similar. It is not common in this country. The eggs infect directly. Raw pork is the common source of infections.

Dibothriocephalus latus, like the two preceding tapeworms, is large. Eggs and segments are both present in the stools. Fish is the source of infection.

Tenia nana is the dwarf-worm. It is found in children and is diagnosed by finding the ova in the stools.

SPUTUM

Introduction.—The saliva and the mucous secretions from the nose, mouth and throat, as well as the material coming from below the larynx, are often spoken of as sputum. For this reason it is important to learn from the patient, if possible, whether the specimen was raised through the larynx by an expiratory effort. To obtain a specimen as free as possible from the nasal and oral secretions it is usually best to collect the material that is brought up through the larynx in the morning.

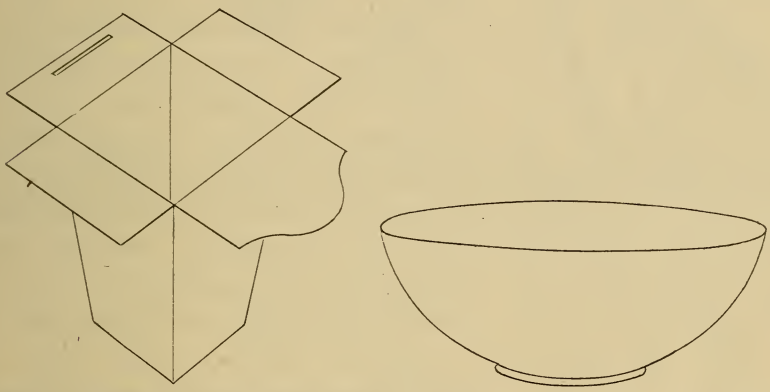


FIG. 16.—SPUTUM. Paper sanitary cup and finger bowl. The cups are lined with heavy paraffined paper. These cups may be obtained from boards of health. The glass finger bowl measures 4.5 inches across the top.

Gross Examination.—Sputum is now usually sent to the hospital laboratory in one of the many varieties of paper cups (Fig. 16) provided for this purpose. The specimen is emptied into a glass finger bowl (Fig. 16) and the empty cup filled with 10 per cent. formalin. The sputum is then examined in the finger bowl against a black table top for gross structures. Pin-head to larger caseous masses of a yellow to white color may be found in tuberculous sputa. In non-tubercular cases of putrid bronchitis similar masses made up of masses of organisms and fat crystals may be found (Dittrich's plugs) (Fig. 18). Casts of bronchioles are found in pneumonic sputum as threads 2 mm. thick and a few centimeters long (Fig. 18). Casts of bronchi are found in fibrinous bronchitis. They often present a tree-like structure

measuring several inches (Fig. 17). Curschmann's spirals (Fig. 18), sometimes found in bronchial asthma, are about the size of the casts of the bronchioles, but when placed under the microscope they show a distinct central spiral arrangement. After completing the examination fill the dish with 10 per cent. formalin.

Fresh Microscopic Preparations (Fig. 19).—At the time of making the gross examination it is often desirable to examine certain of the gross particles under the microscope. To do this the desired material is removed from the dish with a platinum loop and transferred to a slide and a square cover slip applied. These fresh preparations are later dropped into the formalin solution. Red blood corpuscles (if from a fresh hemorrhage they are normal in appearance, are arranged in rouleaux), white cells (pus cells, eosinophiles or endothelial leucocytes), epithelial cells (the pavement variety usually from the nose and throat), and elastic fibrils (to test treat with 10 per cent. KOH) may be recognized. Charcot-Leyden (bronchial asthma), hematoidin, cholesterin and fatty acid crystals may also be identified.

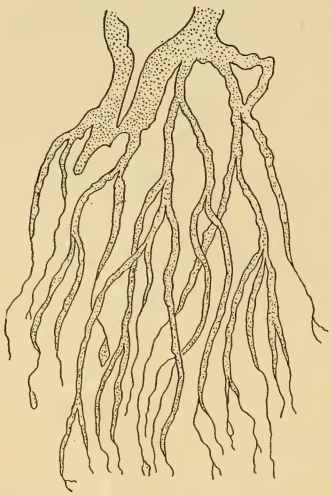


FIG. 17.—SPUTUM. Bronchial cast.

Stained Preparations.—Slides are best for making sputum preparations. An examination for tubercle bacilli is the most common examination asked for, but it is often required to examine for pneumococcus, bacillus influenzæ, bacillus mucosus capsulatus and other organisms as well as for red blood corpuscles, pus cells, and other cells.

ZIEHL-NEELSEN STAIN.—*Tubercle Bacilli.*—With a platinum loop cover three-fourths of a slide with selected particles of the sputum. Warming the slide slightly over a flame facilitates the spreading out of a tenacious sputum. After drying the preparation apply the stain: (1) carbol-fuchsin for two minutes gently steaming;

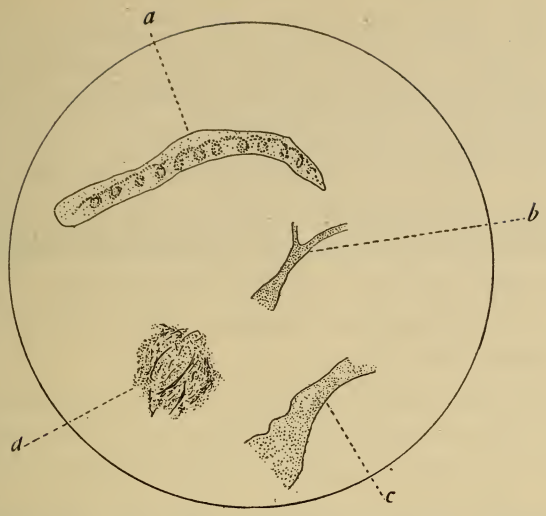


FIG. 18.—SPUTUM. Pathologic macroscopic findings. Examine with naked eye. Identify under low power of microscope. *a*, Curschmann's spiral; *b*, cast or plug from bronchiole; *c*, plug from bronchus; *d*, caseous mass.

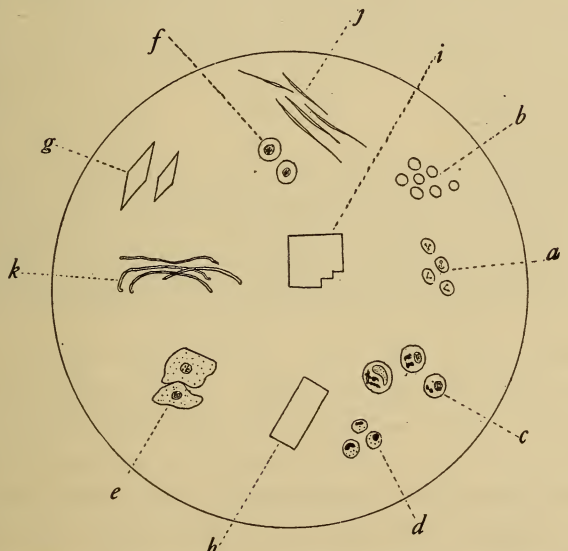


FIG. 19.—SPUTUM. Pathologic microscopic findings. Examine with high dry or oil in fresh and stained preparations. *a*, Neutrophiles (pus cells); *b*, red blood corpuscles; *c*, endothelial leucocytes with blood pigment (heart-failure cells); *d*, eosinophiles; *e*, squamous epithelial cells from the upper air passages; *f*, round epithelial cells from the air sacs; *g*, Charcot-Leyden crystals; *h*, hematoidin crystal; *i*, cholesterin; *j*, fatty acid crystals; *k*, elastic fibrils.

(2) wash with water and then with Czaplewsky until thinner portions of smear are faint pink (about one-half minute); (3) wash with water and counterstain (one-half minute) with Loeffler's methylene blue; (4) wash with water, dry in air and examine with oil immersion. (For the preparation of the solutions, see p. 80)

McJunkin's Polychrome Stain for Blood.—The cells and other bacteria are best stained by covering thin smears with the stain for one-half minute, and then diluting with 2 parts of distilled water and staining five minutes. Wash with distilled water, dry and examine with oil.

Eosinophiles are most often found in bronchial asthma. Endothelial leucocytes containing large amounts of hemosiderin (heart-failure cells), are often present in chronic valvular disease, especially mitral stenosis. In these cases hemosiderin-containing leucocytes are found in the alveolar walls and in the bronchial lymph nodes as well as in the air sacs.

Gram's stain may be necessary to aid in the differentiation of the bacteria found in sputum (see p. 79).

BLOOD

Introduction.—Apparatus for collecting blood for the estimation of hemoglobin, for the determination of the number of red blood corpuscles and white blood cells, and for making smears of the blood, is carried to the bedside of the patient. For examination of patients in their homes this apparatus may be placed in the hemocytometer case (Fig. 20). Provide the pipettes with tips made from small soft rubber corks. Place in the case a straight surgical cutting needle attached to a cork inserted in a small vial containing 95 per cent. alcohol (one of the automatic blood stickers is convenient) and a second small vial containing Hayem's solution. A Tallquist scale removed from a book and cover-glasses may be placed in the case cover.

Hemoglobin.—The Sahli method is practical and fairly accurate, but for close determinations the Miescher method should

be used. The Tallquist scale answers only for rough estimations above 70 per cent.

Tallquist Scale Method.—At the bedside the lobe of an ear or the tip of a finger is wiped off with cotton moistened with alcohol, wiped dry with dry cotton and pricked with the sticker. The

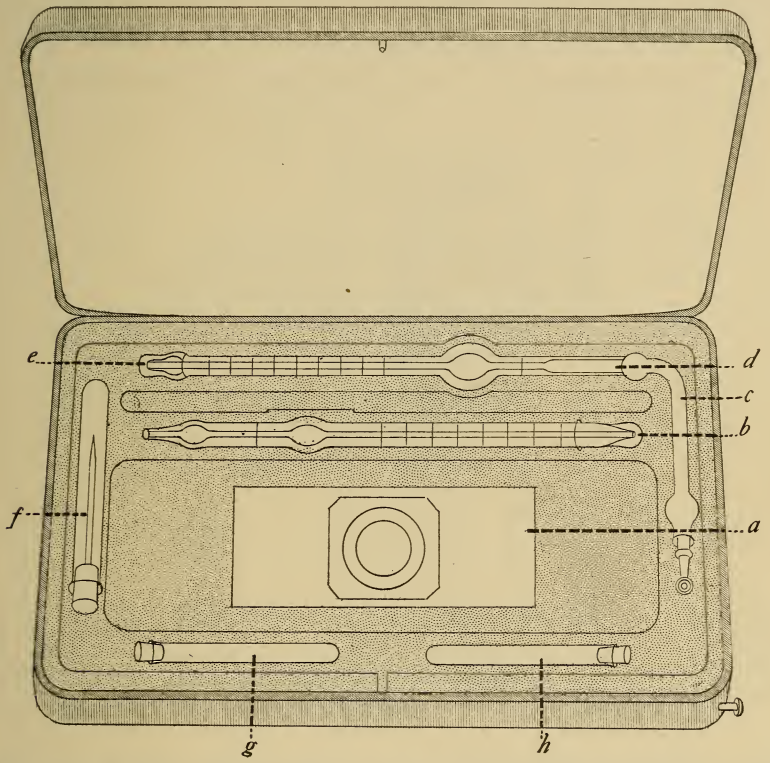


FIG. 20.—BLOOD. Counter case. *a*, Slide with counting-chamber; *b*, rubber cork covering tip of white pipette; *c*, soft rubber tubing; *d*, red pipette provided with rubber cork; *e*, cutting needle in 95 per cent. alcohol; *g*, Hayem's solution; *h*, ½ per cent. acetic acid.

blood must flow freely. The second small drop is allowed to touch the filter paper provided with the Tallquist scale, and as soon as the blood has been absorbed by the paper it is slipped beneath the Tallquist scale and carefully matched. If the hemoglobin is below 70 per cent. a more careful examination by another method should be made.

Sahli Hemometer Method (Fig. 21)—The tube (*b*) graduated from 0 to 140 is filled to 10 with decinormal hydrochloric acid. To the acid in the graduated tube, 20 c.mm. of blood, obtained by filling the pipette (*d*) to the 20-c.mm. mark, are added. Shake and allow to stand exactly one minute. The pipette is rinsed by drawing the blood-acid solution into it. At the end of the minute

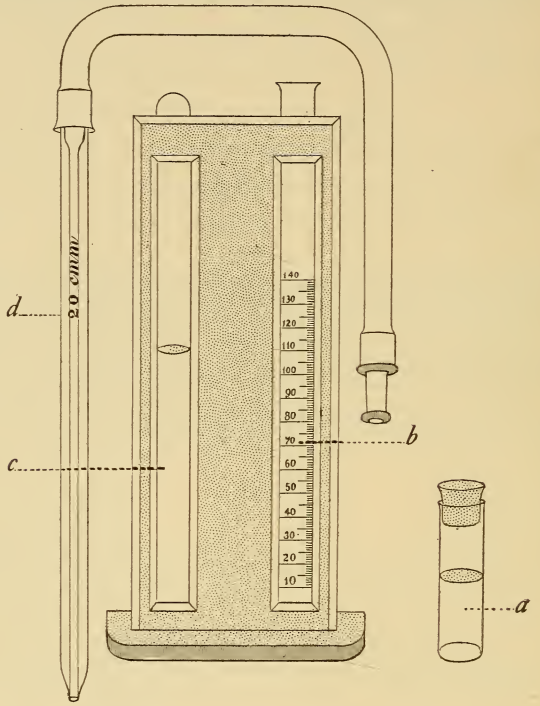


Fig. 21.—Blood. Sahli hemometer. *a*, Vial containing decinormal hydrochloric acid; *b*, graduated tube; *c*, tube with standard solution; *d*, pipette.

water is added until the color matches that in the standard tube (*c*) when the two are placed side by side. The number marking the upper surface of the fluid in the graduated tube is the percentage of hemoglobin.

The tube with the standard solution, contains blood, with a corpuscle count of five million, 1 part, decinormal hydrochloric 10 parts, distilled water 40 parts, and glycerine 50 parts. The solution in this tube should be protected from the light, carefully

stoppered with a cork covered with paraffin, and renewed at the end of each year.

To cleanse the pipette draw through it ½ per cent. acetic acid, alcohol, ether and then air. This may be done by attaching to a suction pump (Fig. 22) or by filling and then expelling the solution by means of a thick rubber bulb. A horse hair may be used to remove any accidental dirt but a wire should never be used. Hairs from the tail of a horse should be kept in

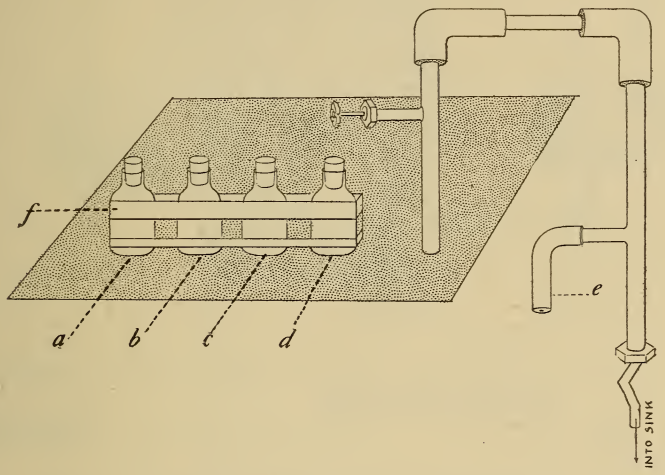


FIG. 22.—BLOOD. Suction apparatus for cleaning pipettes. *a*, Hayem's solution; *b*, ½ per cent. acetic acid; *c*, 95 per cent. alcohol; *d*, ether in 50-c.c. cork-stoppered bottles supported by strips of adhesive or better set into a frame made of thin galvanized iron; *e*, is thick-walled rubber tubing with a 2-mm. lumen into which the upper end of the pipette is inserted.

95 per cent. alcohol for this purpose. Occasionally it may be necessary to fill the pipette with nitric acid and set aside for several hours in this acid to remove albumin from the interior of the pipette.

Miescher Hemoglobinometer Method (Fig. 23).—The blood is drawn up to the ⅔ mark on the pipette (*d*) and then filled to the mark above the bulb with 0.1 per cent. sodium carbonate. Mix and blow out into the half of the cell 15 mm. deep (*a*), filling the other half with water. The half filled with the water is placed above the scale (*e*). Seal the cell with the glass cover (*b*) and then

the metal cap (*c*). The metal cap has a slit which should be placed at right angles to the partition dividing the cell. The instrument is now placed in a dark room before a candle and the red scale (*e*) is slid along until the two colors match when the reading on the scale is recorded. Make five such readings, add them together, and divide by 5. Now transfer the diluted blood by means of the pipette to one-half of the 12-mm. deep chamber which is similar

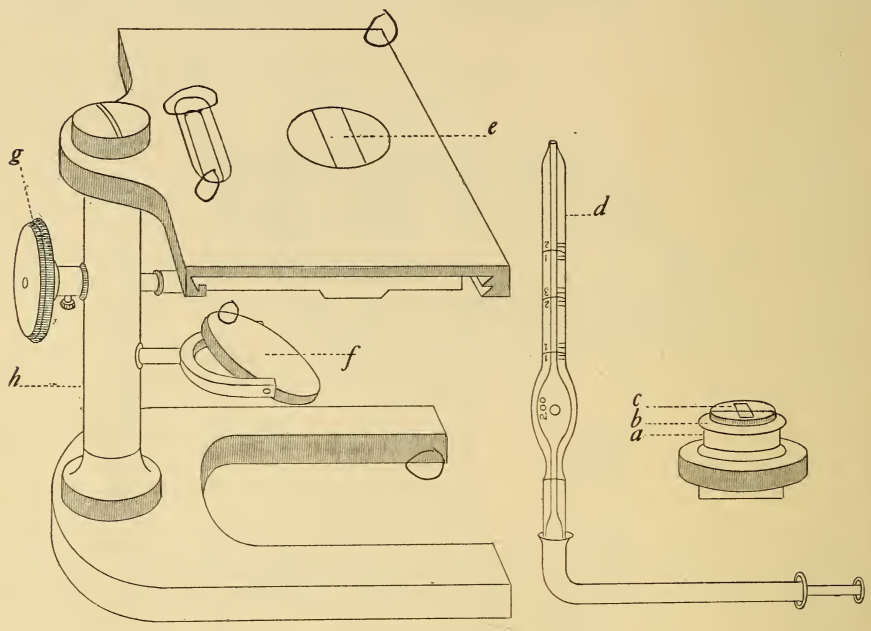


FIG. 23.—BLOOD. Miescher hemoglobinometer. *a*, Cell 15 mm. deep; *b*, glass cover; *c*, metal cover with slit; *d*, pipette; *e*, color scale moved by the thumb screw *g*; *f*, light reflector; *h*, Fleischl modification of the instrument is made by Reichert of Vienna.

to (*a*) and proceed to make five more readings. Add these five readings together, take the average and multiply by $^{15}/_{12}$. Add the two averages together and divide by 2. In cases of anemia, draw the blood up to 1 on the pipette thus obtaining a 1–200 dilution instead of a 1–300. In this case divide the final average by 1.5. To get the percentage of hemoglobin, follow the directions for the scale that comes with each instrument. Clean the pipette as indicated under the Sahli method.

Dare Hemoglobinometer Method (Fig. 24).—The determination of hemoglobin with Dare's hemoglobinometer rests on the comparison of a film of undiluted blood of definite thickness with standardized (*a*) colored-glass disk. The finger or ear is pricked and the slit of the blood chamber (*x*, *w*) when brought into contact

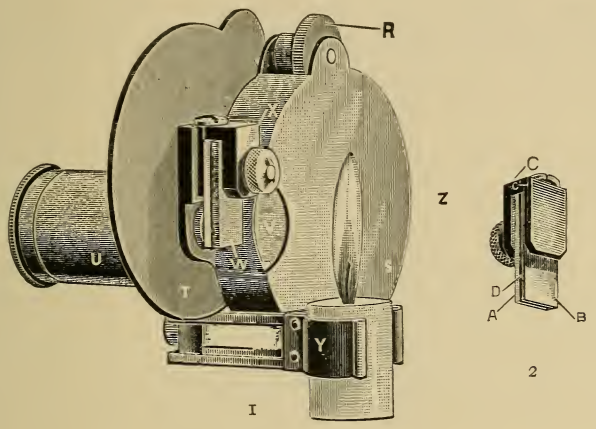


FIG. 24.—1, Dare hemoglobinometer; 2, blood chamber detached.

with the blood immediately fills by capillarity. The excess of blood is removed and the blood chamber replaced in its slot or carrier with the white glass facing out. Light the candle and looking through the eyepiece (*U*) rotate (*r*) until the colors match. The hemoglobin is read directly from the scale. Many prefer to use this instrument as a routine in preference to the Sahli.

Enumeration of the Red Blood Corpuscles (Zeiss Hemocytometer) (Fig. 11).—The pipette marked 101 above the bulb is used in counting the red blood corpuscles, and the one bearing the mark 11 is used in making the dilution for counting the leucocytes. These two pipettes are filled at the time the blood is taken for the hemoglobin estimation.

The red corpuscle pipette is placed in a small drop of blood that has freely escaped onto the skin surface and gentle suction made, using the tongue to shut off a small space in the front part

of the mouth for this purpose. The blood is drawn into the pipette until it reaches the 0.5 mark when the suction is stopped and immediately withdrawn from the drop of blood. If the column of blood reaches more than 1 mm. above the 0.5 then draw on up to 0.6 for the dilution. If the column of blood only reaches about 1 mm. above the 0.5 mark then the excess may be expelled by

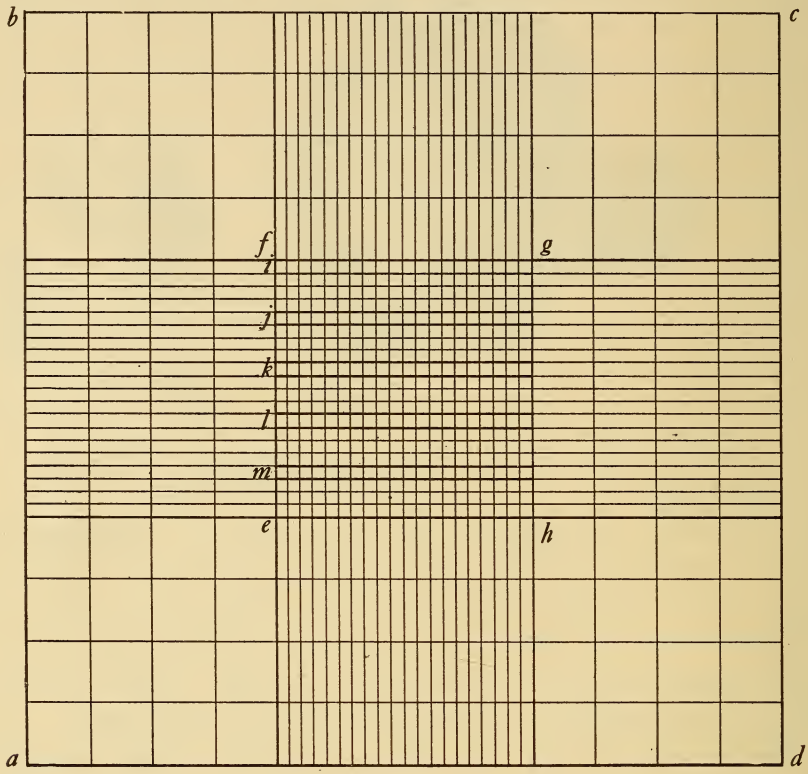


FIG. 25.—BLOOD. Counting chamber. A mechanical stage is necessary for blood work. Counting by strips facilitates the enumeration of the corpuscles. The entire ruled area 3 mm. square is divided into nine equal squares of 1 sq. mm. each.

touching the tip of the pipette very lightly to the adjacent dry skin surface. As soon as the blood has been drawn to the 0.5 mark, place the tip of the vial in Hayem's solution (mercuric chloride 0.5 gm., sodium chloride 1.0 gm., sodium sulphate 5.0 gm. and distilled water 200 c.c.) and draw up to 101. Place the finger over the tip of the pipette, shake one minute and apply the rubber cap

in case the pipettes are to be transported to a laboratory some distance away; if the laboratory is at hand this is not necessary.

To make the count shake again for one minute, force 1 or 2 drops out of the pipette, and let a small drop escape on to the center of the counting-chamber island (Fig. 25) by touching it with the tip of the pipette. Apply the cover-glass and allow the corpuscles to settle. The diluted blood should just cover the island and be free from bubbles of air. When viewed tangentially Newton's rings should appear between the cover and the slide about the moat.

The ruled part of the island (*a b c d*) is 3 mm. square, while the central finely ruled area (*e f g h*) is 1 mm. square. The distance between cover and upper surface of island is 0.1 mm. A side (*e f*) of the central finely ruled square is divided into 20 equal parts. A strip (*i, j, k, l,* or *m*) finely ruled and measuring $\frac{1}{20}$ by 1 mm. may then be used as a basis for the count. Such a strip of course is made up of 20 small squares. At least ten such strips must be counted and it is better to count five in the first preparation, and five from a second preparation made by wiping the diluted blood from the island and replacing it from the pipette. One of these strips has of course a volume of $\frac{1}{10} \times \frac{1}{20} \times 1 = \frac{1}{200}$ c.mm. and the dilution is 1–200. Therefore multiply the average number of red blood corpuscles in one strip by $200 \times 200 = 40{,}000$ to get the number of corpuscles in 1 c.mm.

Other methods of enumerating the corpuscles are used. All the numerous rulings of the counting chamber agree in having a central finely ruled area 1 mm. square. Each side of this central square is laid off into 20 equal parts, thus dividing it into 400 small squares. The small squares of course are $\frac{1}{20}$ mm. square.

In a method extensively employed the small square ($\frac{1}{20} \times \frac{1}{20}$ mm.) is used as a basis. The depth of the blood between the island and the under surface of the cover is $\frac{1}{10}$ mm.; therefore the volume of blood above each small square is $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ or $\frac{1}{4000}$ c.mm. The dilution is 1–200. Count the number of corpuscles in 80 small squares and obtain the average in each square. Multiply the average by 800,000 (contents × dilution)

to get the number of corpuscles in 1 c.mm. of undiluted blood. Example: 480 corpuscles in 80 squares = 6 cells as an average. 6 × 800,000 = 4,800,000 corpuscles in 1 c.mm. Since we divide by 80 and again multiply by 800,000, the number of corpuscles in 80 squares may be counted and four ciphers annexed to this number.

To cleanse the counting chamber thoroughly wipe off with a soft dry cloth. The pipette is cleansed as in the case of the Sahli pipette (p. 35).

Enumeration of the White Blood Cells.—The blood is drawn into the white pipette to 0.5 and this diluted with ½ per cent. acetic acid (a stock bottle containing a crystal of thymol is kept on hand) to the 11 mark. Fill the counting chamber (Fig. 25) as in the case of the red blood corpuscles and count all the leucocytes in the 9 sq. mm. To obtain the number of leucocytes in 1 c.mm. multiply the number in 1 sq. mm. by 200 (contents × dilution).

To facilitate the counting, count all corpuscles or cells touching left-hand and upper borders. Do not count any corpuscles that touch the lower or right-hand borders. For example, although a corpuscle lies entirely without the area being counted, but barely touches the upper border, it is enumerated.

In counting the corpuscles use the high dry objective and in counting the white cells use the low dry. There should be four to six million red blood corpuscles per cubic millimeter while the leucocytes have a normal variation of from five to ten thousand. To obtain the color index divide the percentage of hemoglobin by the percentage of corpuscles (5,000,000 corpuscles = 100 per cent.). If the index is below one the individual corpuscle is poor in hemoglobin.

Coagulation Time of the Blood (Bogg's Coagulometer) (Fig. 26).—A small drop of blood is placed on the lower surface of the conical glass disk (*a*) and the disk inserted into a moist chamber (*b*). Place under low power of the microscope and bring the drop into focus. When clotting has taken place gentle pressure on the rubber tube (*c*) after pinching off the end of the tubing moves

masses of the corpuscles but they spring back as an elastic mass. The normal coagulation time is three to eight minutes.

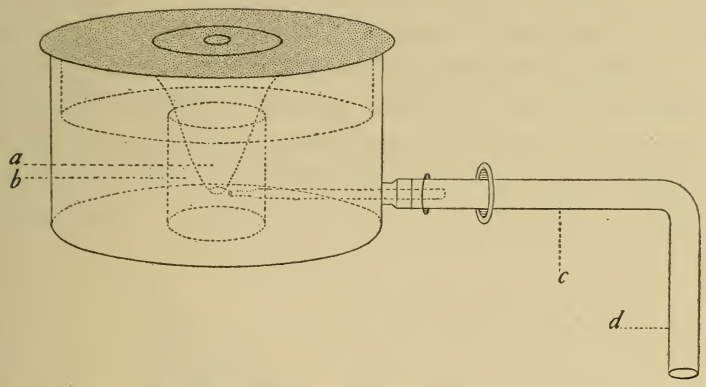


FIG. 26.—BLOOD. Coagulometer. *a*, Conical glass disk; *b*, moist chamber. Compress the rubber tubing at *c* after pinching off at *d*.

Blood Films.—Cover-glasses (No. 1, 22 mm. square) are placed in concentrated sulphuric acid over night, when, after removing all acid by washing out under the tap, they are covered with 95 per cent. alcohol and finally transferred to chloroform. The covers are removed from the chloroform with forceps and wiped dry between two small boards covered with boiled muslin so as not to bring the fingers into contact with the covers. Place in box or glass dish with forceps. The removal of all fat is an absolute requirement if suitable films are to be obtained.

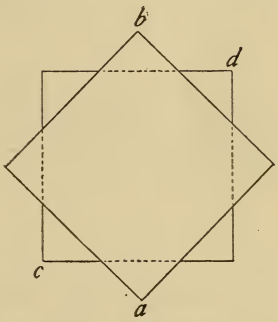


FIG. 27.—Blood film by the cover-glass method. With thumb and forefinger catch the upper cover-glass (22 mm. square) at *a* and *b* and the lower one at *c* and *d*.

Touch one fat-free cover at its center to the ear so as to pick up a drop of blood the size of a pin-head and apply a second cover to it so that the corners may be grasped (Fig. 27). As soon as the blood has spread out between the covers pull them apart, being careful to keep the two parallel. If slides are used the best smears are secured by spreading a small drop on a fat-free slide as shown in Fig. 28. The cover-glass method is preferable.

Differential Counting of the Leucocytes.—The films when dry are stained with a polychrome blood stain. The polychrome blood stains differ from the eosin-methylene blue stains in that the nuclei stain reddish while the cytoplasm tends to stain bluish. Many of the commercial polychrome blood stains give only the eosin-methylene blue staining. In the McJunkin's polychrome stain for blood (obtained from Bausch and Lomb Optical Co.), the reaction is accurately adjusted by titration and a definite polychrome staining is secured.

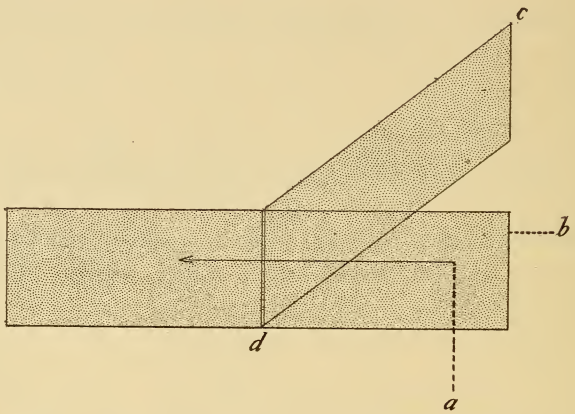


FIG. 28.—BLOOD. Blood film by the slide method. Place a drop of blood twice the size of a pin-head on a fat-free slide *b* at a point *a*. Place the end of a second slide *c* in it, and after the drop has spread along the entire surface of contact *d*, the film is made by pushing *c* in the direction shown by the arrow.

Two drops of the stain are placed on the cover-glass held in suitable forceps (Fig. 29) for one-half minute so as to fix the film. The stain is then diluted with 4 drops of distilled water and the diluted stain allowed to act for five minutes. Wash with distilled water for a few seconds, dry and mount in colophonium-xylol. If the preparation has been made on a slide (Fig. 29), cover with 8 drops of the stain for one-half minute, dilute with 16 drops of distilled water and stain five minutes. Wash, dry and examine with the oil immersion.

The three varieties of leucocytes of the normal blood, lymphoblastic, myeloblastic, and endothelial are well differentiated after this technic. The classes to be tabulated in the differential count

are lymphocytes (22), neutrophiles (72), immature neutrophiles (4), eosinophiles (1.5), basophiles 0.25 and endothelial leucocytes 0.25. There is doubtful value in attempting to divide the lymphocytes into large (young) and old (small). The numbers given indicate normal counts that may be obtained. In counting it is necessary to include all cells up to the very margin of the film regardless of the method used in making the film. This precaution is especially important, however, in the case of films made on slides.

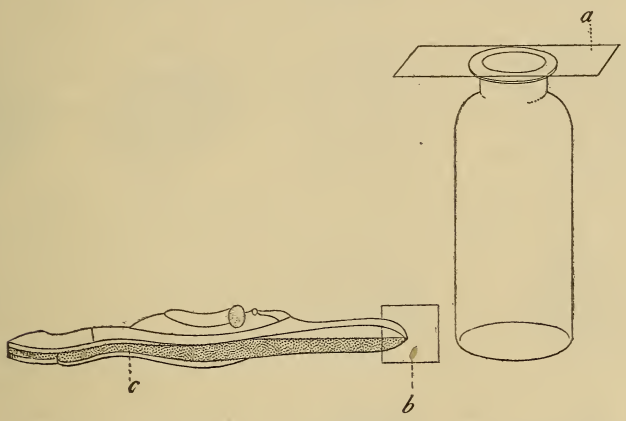


FIG. 29.—BLOOD. Staining of cover-glass and slide preparations. *a*, 3 by 1 extra white, medium thickness, and fat-free slide supported by a small bottle to prevent the alcoholic stain from running over the sides; *b*, 22 mm. square, number 1, fat-free cover-glass; *c*, Novy cover-glass forceps. *a*, *b* and *c* may be obtained from Bausch and Lomb.

Lymphoblastic Cells (*A*, Fig. 30).—These cells come from the cells of the germinal centers of lymph nodules and under certain conditions (lymphatic leukemia) the young form of cell may be found in the peripheral circulation. The cytoplasm and the nucleus of these young cells stain less intensely than the same structures in the older forms, but the cells are otherwise like the lymphocytes. Very rarely plasma cells may be found in the peripheral blood.

The very young lymphoblastic cells average smaller than young myeloblastic cells; the former do not give the oxydase reaction. This reaction is carried out as follows: (1) Fix in formalin; (2)

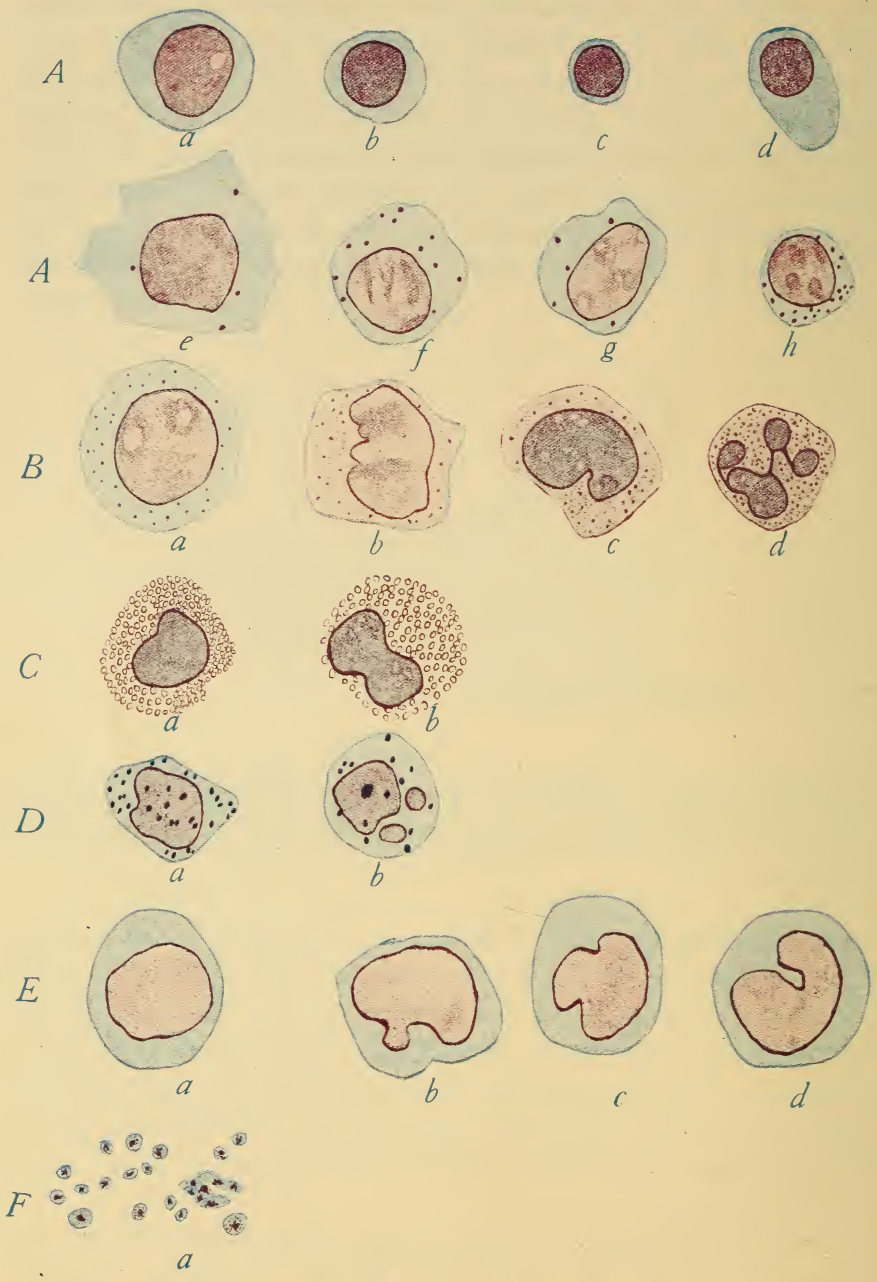


FIG. 30.

place in a 1 per cent. solution of alpha-naphthol in 1 per cent. potassium hydrate for five minutes; (3) transfer to 1 per cent. aqueous solution of dimethyl-*p*-phenylenediamin (Merck) for five minutes; (4) wash and mount in glycerine jelly. The myeloblastic cells are bluish (Schultze).

The identification of the lymphocyte depends largely on negative properties. As in all blood work the stain used must be a good one and its staining characters known in detail. The nucleus, as in the identification of all cells, is of less importance than the cytoplasm. The cytoplasm of the lymphocyte is a robin's egg blue if much spread out, or a dark blue if the rim of cytoplasm about the nucleus is narrow. The cytoplasm is usually entirely homogeneous but often nodal points of a heavier blue are

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FIG. 30.—BLOOD. Normal leucocytes of the peripheral blood stream. These cells are stained with a polychrome blood stain and drawn on the same scale with the aid of a camera lucida. Some of the variations in size are unquestionably the result of the smearing out of the blood.

A. Lymphocytes. *a*, Young lymphoblast drawn from a smear from a case of acute lymphatic leukemia. This cell is rarely found in normal blood. *b*, Lymphocyte; *c*, older lymphocyte corresponding to the lymphoid cells of the tissue; *d*, plasma cell, rarely seen in the peripheral blood. This cell is reproduced from a smear from a case of chronic appendicitis. *e*, *f*, *g*, and *h* are lymphocytes that contain "azur" granules. These cells are large and their cytoplasm is not dense. In some cases at least as in *e*, the apparent large size has resulted from the smearing out of the blood.

B. Neutrophiles. *a*, Neutrophilic myelocyte drawn from a smear from a case of chronic myelogenous leukemia. This cell is not found in normal blood. *b* and *c*, Young (immature) neutrophiles. These cells have neutrophilic granules, and in cases where there is a neutrophilic leucocytosis the percentage of these varies greatly. At present it seems advisable to place them in a separate class. *d*, Neutrophile. The aging of *b* and *c* into *d* is seen not only in the blood of myelogenous leukemia but also in normal blood.

C. Eosinophiles. *a* with its larger and less broken nucleus is a younger cell than *b*. The granules are very coarse and in the best preparations are not intensely stained. A stain that colors these granules an intense red is not a good polychrome stain.

D. Basophiles. *a* is a younger cell than *b*. The coarse granules take a purplish heavy stain.

E. Endothelial Leucocytes. In size and morphology, these cells may closely resemble the neutrophiles *b* and *c*, but the cytoplasm is entirely free from reddish granules and stains a heavier blue. *b*, *c*, and *d* have the non-granular basophilic cytoplasm in which the nodal points are very apparent. These cells are rather uncommon (below 0.25 per cent.) in the normal peripheral blood. They should be studied in preparations from cases of typhoid fever before an attempt is made to recognize them in normal blood.

F. Blood Platelets. These are detached portions of megakaryocytes. Note the central purplish granular portion and the peripheral blue portion.

apparent, and if the preparation is grossly overstained these nodes may appear as reddish granules thickly peppered in.

In the usual cover-glass and slide smears, from 30 to 80 per cent. of lymphocytes shows scattered rather coarse reddish granules usually under twelve in number. The cells showing the granules are usually large with an abundant light-staining cytoplasm.

These granules in the cytoplasm of lymphocytes have usually been called "azur granules." They stain like the nucleus and appear to be particles of nuclear material. A certain number of them at least are artefacts, produced by rubbing off small particles of nucleus in making the smears. This is shown by the increase in the percentage of lymphocytes showing these granules in very thin films of the same blood. It is widely stated that the lymphocytes of lymph nodes do not show these granules, but preparations made so thin that the lymphocytes are the same size that they are in the blood films, show lymphocytes with "azur granules." These cells have the other characters of lymphocytes; they do not phagocyte in experimental animals *in vivo* and in the human blood they do not phagocyte *in vitro*. They have the para-nuclear granules described by Schridde. The larger of these lymphocytes with light-staining abundant cytoplasm and "azur granules" have by some been called "large mononuclears." As noted above this abundant light staining cytoplasm is due to the pressing out of the cells on the glass.

Myeloblastic Cells (*B*, *C*, *D*, Fig. 30).—The young cells of this series found normally only in the bone marrow are microscopically much like the young lymphoblasts. They average somewhat larger in size, give the oxydase reaction and do not show the Schridde granules. However, one of three varieties of granules soon appears in the cytoplasm. A transition form between the youngest parent myeloblast of the bone marrow and the mature neutrophile, eosinophile and basophile of the blood is known as a neutrophilic, eosinophilic, or basophilic myelocyte. The recognition of the last two offers no difficulties. In fact the cytoplasm of the mature myeloblastic cells possesses so typical an appearance due to the presence of granules that there is no difficulty in classifying them. The development of these mature cells from non-

granular young cells may be so conclusively followed in the bone marrow that there is now no question about the origin in the adult of these cells.

In general, immature or young cells owing to their partial differentiation are more difficult to identify. The young forms of the eosinophile and basophile are easily recognized by the large size and characteristic staining of their respective granules. The granules of the neutrophiles are finer and much less conspicuous than eosinophilic and basophilic granules. Thus in the young neutrophiles as would be supposed the granules are imperfectly developed and the demonstration of these requires the use of a stain with an accurately adjusted reaction. The nucleus of such young neutrophiles varies between the two extremes, the round nucleus of the neutrophilic myelocyte and the nucleus of the mature neutrophile, that is, the nucleus may be identical with the nucleus of the endothelial leucocyte and these two cells have been extensively confused, usually under the term "transitionals." To avoid such confusion neutrophile granules must be demonstrated in the young (immature) neutrophiles.

The Endothelial Leucocytes (*E*, Fig. 30).—These cells arise from the endothelial cells of the blood- and lymph-vessels. These leucocytes as found in the blood stream average distinctly larger than the mature neutrophile. In certain pathologic processes, for example typhoid fever, these cells may be seen becoming free definitely within a blood capillary following a mitosis. The nucleus of these cells may be round, but it is more often indented or horseshoe in shape, that is, it resembles the nucleus of the immature neutrophile. The cytoplasm, however, is free from granules and this differentiates it with certainty from young neutrophiles. In properly stained specimens the identification of the young neutrophile rests entirely on the presence of neutrophile granules. The cytoplasm of the endothelial leucocyte is blue and the blue modal points are very distinct. This gives the cytoplasm a richly staining appearance with nodal points. It does not have the homogeneous appearance of the lymphocyte. The cytoplasm of the young neutrophile has a tinge of blue that varies in degree with the age of the cell. The endothelial leucocyte

should be studied in smears from typhoid fever cases before attempting to identify it in normal blood. Counts recently made on eleven typhoid fever cases show an average of 2.4 per cent. of these cells.

Red Blood Corpuscles.—They stain an orange or pink. Microcytes have a diameter of less than 7.5 microns, normocytes measure 7.5 microns while the macrocytes are abnormally large. Poikilocytes are irregularly shaped corpuscles. Stippling is a term applied to a basophilic granulation of the corpuscle. If due to lead, a reaction is obtained on treatment with an alkaline sulphide.[1] Polychromatophilia is a bluish staining of the corpuscle.

Normoblasts are nucleated reds, with a diameter of 7.5 microns, microblasts are small nucleated red cells, while megaloblasts are nucleated reds measuring above 15 microns. The nuclei of nucleated reds are dense, round and often eccentric and the cytoplasm stains an orange to a blue.

Platelets (*F*, Fig. 30).—These elements, about one-third the diameter of a corpuscle but often elongated, are detached portions of megakarocyte pseudopoda. They are bluish with purplish granules toward the center when stained with a polychrome blood stain. There are about a half million per cubic millimeter, and perhaps the best method of estimating their number is by comparison with the corpuscles in properly stained smears on cover-glasses.

Protozoa in Blood Films.—At the present time malarial parasites are of first importance. The McJunkin's polychrome stain for blood is very satisfactory for the demonstration of these parasites, the chromatin staining red. In order to stain the parasites heavily for the study of structure, the author's polychrome stain for protozoa should be used.

McJunkin's Polychrome Stain for Protozoa (obtained from Bausch & Lomb Optical Co.).[2]—This stain is readily prepared; 1 gm. of methylene blue (Grubler's B. X.), 50 c.c. of decinormal (no factor) sodium carbonate and 50 c.c. of glycerine (Merck U. S. P.) are placed in a 500-c.c. beaker and heated at 87° to 89°C.

[1] See Jour. Med. Research, May, 1915, page 271.

[2] See Jour. A. M. A., Dec. 18, 1915, page 2164.

for one hour, constantly stirring with a mechanical stirrer (Fig. 31). The carbonate solution that has been made decinormal by titration with a standard acid using methyl red as an indicator must be run in accurately from a burette. The heating is best carried out by placing the beaker in a water-bath regulated at 94° to 96°C. Remove the beaker from the water-bath and pour the polychrome solution while hot ino a 100-c.c. graduate. Rinse the beaker out with 5 c.c. of distilled water to remove any carbonate and add this to the contents of the graduate.

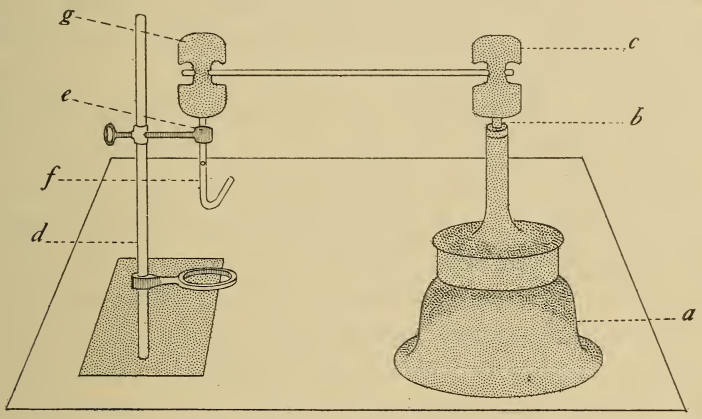

FIG. 31.—BLOOD. Mechanical Stirrer. *a*, Small water or electric centrifuge; *b*, axle carrying the head in which the centrifuge tubes are placed. This head is unscrewed from the top of the axle and the wooden pulley *c* screwed on. *c* has one deep groove and should have a shallower one also. *d*, burette stand; *e*, small cylinder of wood through which runs a short metal tube. The lower end of this metal tube carries the glass stirrer *f*, while the upper end carries the pulley *g*.

Into a second graduate, measure sufficient methyl alcohol (Merck Reagent or Kahlbaum acetone free) to make 100 c.c. when added to the polychrome solution. Pour the alcohol into a 4-ounce bottle and add 0.75 gm. methylene blue (Grübler's B. X.) and 0.25 gm. eosin (Grübler's yellowish, water soluble). After complete solution of the methylene blue and the eosin is secured by shaking, pour the polychrome solution into the bottle. The total volume is now 100 c.c. If the solution is not complete place in the paraffin oven at 52°C. one hour or more.

To use add 1 drop of the stain to 1 c.c. of distilled water and

float the cover-glass preparation that has been fixed for ten minutes or more in equal parts of alcohol and ether or in methyl alcohol, on the surface of the diluted stain for thirty to sixty minutes. Wash with distilled water for one to five minutes, air dry and mount in colophonium-xylol.

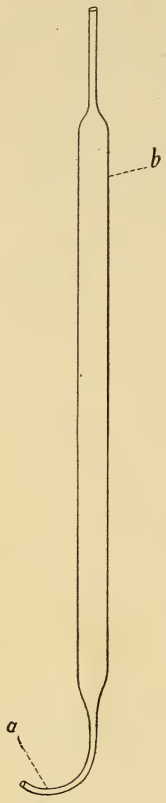

FIG. 32.—BLOOD. Wright pipette. Fill by touching the curved end *a* to a large drop of blood. Seal at *a* in a flame. After clotting the tube may be scratched at *b* with a file, broken and the serum removed with a capillary pipette.

Occasionally a sample of Grübler's yellowish, water-soluble eosin is encountered that cannot be used. If the stain is made with such an eosin, red blood corpuscles stain a blue that cannot be washed out. Once an eosin is found that is satisfactory it should be kept for this purpose.

McJunkin's Polychrome Stain for Blood (obtained from Bausch & Lomb Optical Co.).[1]—To prepare McJunkin's polychrome stain for blood, add 250 c.c. of methyl alcohol (Merck reagent or Kahlbaum acetone free), 0.25 gm. of eosin (Grübler's yellowish, water soluble), and 2½ c.c. of half-normal hydrochloric acid to 50 c.c. of the polychrome stain for protozoa. The half-normal acid is added accurately from a burette. Apply according to the directions for the differential staining of leucocytes (p. 42).

Widal Reaction.—A Wright pipette (Fig. 32) is filled with blood from the patient's ear and the serum allowed to separate out. One part of this serum and 29 parts of normal saline measured in a capillary pipette are mixed in the well of a concave slide. One part of this dilution and one part of a twelve-hour bouillon culture of typhoid bacilli are mixed and a hanging drop preparation made from the mixture. If positive there should be agglutination in one hour. A control hanging drop without the serum is always made.

Phagocytosis in Vitro.—To determine the ability of leuco-

[1] See Jour. A. M. A., Dec. 18, 1915, page 2164.

cytes to incorporate foreign bodies in their cytoplasm, it is necessary to prepare in a prescribed way the three ingredients required in the reaction.

Leucocytes.—First the leucocytes are prepared by placing 4 c.c. 2 per cent. sodium citrate in a 15-c.c. graduated centrifuge tube and adding 1 c.c. blood from a finger or ear. Shake, make up to 15 c.c. with normal saline and centrifuge. Pipette off the clear supernatant fluid, again add normal saline to 15 c.c. and centrifuge. Pipette off again, add saline to 1 c.c. and shake. This is the preparation of leucocytes.

Serum.—To enable leucocytes to take up actively foreign particles, the presence of fresh unheated serum is required. The substances present in the serum that enable the leucocytes to act in this way have been named opsonins. At the time the 1 c.c. of blood is taken from the finger or ear for the leucocytes a small Wright pipette is filled with blood which is allowed to clot. One end of the pipette is then sealed, the other end is broken off and with a minute glass thread, the clot is stirred up. This tube is placed in the centrifuge along with the preparation of leucocytes.

Suspension of the Foreign Body.—This is usually a suspension of bacteria. Bacteria such as staphylococcus, streptococcus, or typhoid bacilli are grown for eighteen hours on an agar slant, the tube is half filled with normal saline and the growth rubbed off into the saline with a platinum loop. The suspension is pipetted off into another cork-stoppered tube and shaken in a mechanical shaker for an hour.

To make the test thoroughly mix in the well of a slide equal parts of the leucocytes, serum and bacterial suspension. Use a capillary pipette for measuring. Place the pipette containing this mixture at 37°C. for fifteen minutes. Make smears by the slide method. Stain with a polychrome blood stain and count the number of bacteria in 100 leucocytes. The average number of bacteria per leucocyte is the phagocytic index of the individual whose serum is used. The phagocytic index of a patient divided by the phagocytic index of a normal (control) individual is the so-called opsonic index.

Blood Cultures.—To make a blood culture carefully, not only is the inoculation of media in tubes and flasks usually required, but also the plating of the blood. As a result this part of the

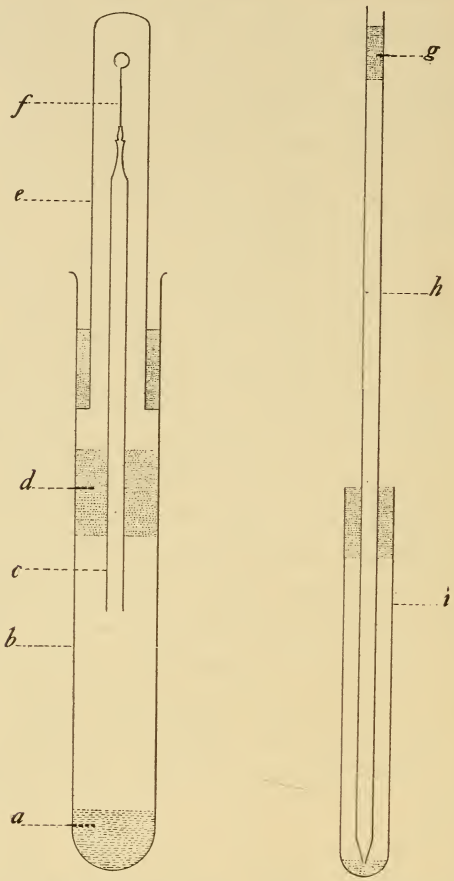

FIG. 33.—BLOOD. McJunkin's blood tube. *a*, Oxalate solution; *b*, extra large test-tube; *c*, 3-mm. soft rubber tubing; *d*, cotton; *e*, small test-tube; *f*, large needle provided with a large stilette; *g*, cotton; *h*, 10-c.c. pipette; *i*, small test-tube.

routine of a hospital laboratory is time consuming and laborious, chiefly for the reason that much apparatus must be carried to the bedside, where the inoculations are always made under certain difficulties.

In the collection of blood for Wassermann tests, in which per-

fect asepsis is not obligatory, a needle is inserted into the arm vein and the blood run directly into a sterile tube. It is the ease and speed with which a large number of specimens of blood may be collected for this test that prompted the use of a tube with oxalate solution in it and a needle attached.

McJunkin's Blood Tube[1] (Fig. 33).—The preparation of the tube is simple. In an extra large test-tube there are placed 15 c.c. of a solution (*a*) which contains 2 gm. of ammonium oxalate and 6 gm. of sodium chloride to a liter of distilled water. Cotton 4 cm. wide (*d*) is now wrapped around the rubber tubing (*c*), which is 150 mm. long with a 1-mm. wall and 3-mm. lumen, and into the upper end of this tubing there is inserted a 19-gage needle 1 inch long (*f*). The needle is capped by inserting into the upper end of the extra large tube a smaller one (*g*) about the lower end of which is wrapped a 3-cm. plug of cotton. Both cotton plugs should fit snugly. The tube complete is autoclaved for twenty minutes at 110°C.

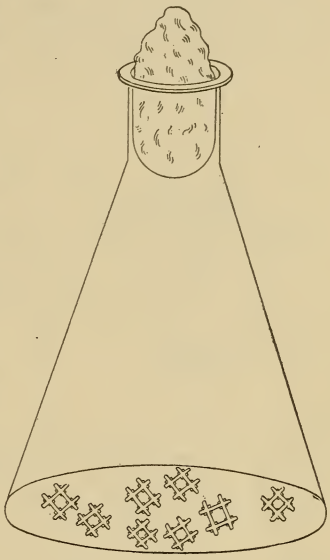

Fig. 34.—Blood. Erlenmeyer flask for defibrinating blood. 125-c.c. sterile flask containing a dozen bits of fine mesh, well-galvanized wire gauze. Shake by hand or place in defibrinator, Fig. 35.

After the needle has been inserted into the vein, a few seconds are required for the blood to pass through the tubing. The lower cotton plug eliminates all chances of contamination and when it is removed in the laboratory, the upper end of the tube is flamed and the diluted blood transferred with a 10-c.c. sterile pipette (*h*) to the media that are indicated. The tubing and needle are cleansed by forcing through them a few cubic centimeters of water from a syringe, after which they are placed in a quart Mason jar in a saturated solution of borax.

[1] *Jour. A. M. A.*, Mar. 7, 1914, page 774.

Inoculation of Media.—In suspected cases of typhoid a flask of bouillon (p. 75) and a flask of lactose bile are inoculated with several cubic centimeters of blood. On the following day and each succeeding day, a transplant is made from each of the flasks to an agar slant and a tube of litmus milk. If the agar slants show a growth of Gram negative bacilli and the milk is not changed, an agglutination test with a known typhoid serum is made.

To obtain the agglutinating serum a rabbit is given five intraperitoneal injections of the typhoid bacilli at three- or four-

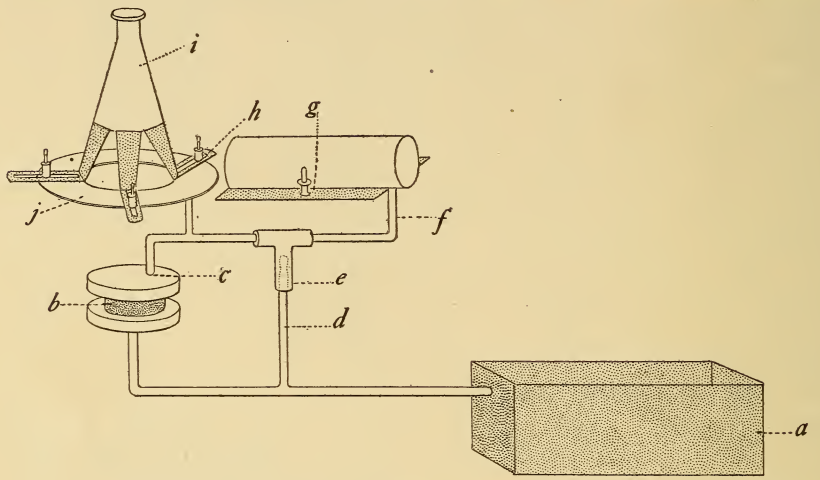

Fig. 35.—Blood. Mechanical defibrinater and shaker (Barta). *a*, Heavy wooden block to be nailed onto platform at desired place; *d* is a ⅛-inch iron rod firmly driven into block and supporting wooden pulley, *b*, and eccentric support, *c*. *b* is run from pulley shown in Fig. 29 by a cord; *f* is a ⅛-inch iron rod attached to the wooden pulley eccentrically and running through the T-shaped tube *e; g*, *j* and *h* are thin sheet iron; *g* and *j* are attached firmly. Tubes to be shaken are clamped into *g* while flasks are clamped into *j*.

day intervals. On the first day the animal is given a suspension of one-half an agar-slant culture. To prepare this suspension 10 c.c. of sterile saline is added to the slant, the growth rubbed off with a platinum loop and the tube placed in water at 60°C. for one hour. At the second and third injection give one tube and at the fourth and fifth injections two tubes. Bleed from the carotid ten days after the last injection. Agglutinating sera other than typhoid (dysentery and the paratyphoids especially) should be kept on hand.

In cases other than typhoid, three glucose agar plates are poured. One flask of bouillon, one tube of lactose bile and one tube of litmus milk are inoculated as a routine. Other media are inoculated if indicated. Each day for at least five days the liquid media are examined and transplants are made to agar slants. The plates are examined daily and are kept for at least ten days. Transplants are made to the various special media as indicated.

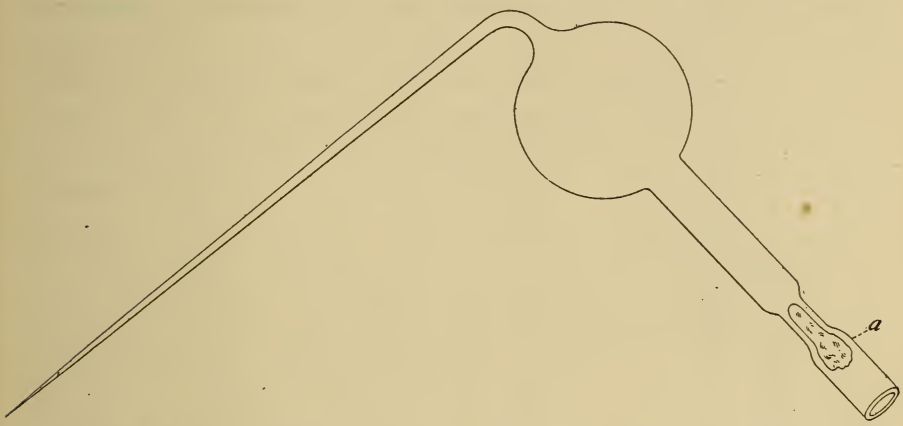

FIG. 36.—BLOOD. Bulb pipette. Secure soft glass tubing for blowing with 8 mm. outside and 4 mm. inside diameter. Cut into 30-cm. pieces and constrict both ends *a*, and plug with cotton. Dry-heat sterilize. Heat the central portion and draw apart into two pipettes. Blow bulbs of desired size and then bend the pipette at a convenient angle. Such pipettes are used for transferring sterile fluids.

With the usual technic, contaminations are rather common. For this reason the examiner must consider all possible data so as to determine whether the organism present came from the patient's blood. If there is no growth of pathogenic organisms on any of the media at the end of ten days the culture is reported as negative.

Wassermann Test.—Introduction.—This test is based on the complement fixation reaction. The antigen is not specific and the reaction is not absolutely specific for syphilis. Unless the clinician is thoroughly familiar with the examiner's technic it is advisable to provide him with the data contained in this and the following paragraph. A positive reaction not due to syphilis may be obtained in yaws, trypanosomiasis, and leprosy and some

have observed it rarely in scarlet fever, malaria, lobar pneumonia, diabetes mellitus and immediately after ether anesthesia. A negative reaction in cases of syphilis may be obtained during treatment with salvarsan or mercury and is said to occur during acute alcoholism.

In negative reactions all the corpuscles are hemolyzed, while in perfect positive ones there is no hemolysis. Between these two extremes, all degrees of hemolysis occur. Those that show only a slight clouding are marked "positive +," which represents less than 10 per cent. inhibition of hemolysis. Such a result has no diagnostic value except in cases of known syphilis under treatment. In such cases further active treatment is indicated. A greater clouding, but one in which there is distinct hemolysis in the clear liquid above the sedimenting corpuscles, represents an inhibition of hemolysis varying between 10 per cent. and 90 per cent., "positive ++." Such a result should be considered as a positive diagnosis of syphilis only in connection with strong clinical evidence. It calls for further active treatment. In cases where there is no tinge of red in the liquid above the sedimenting corpuscles, there is complete inhibition of hemolysis, "positive +++." Such a result is to be considered as a positive diagnosis of syphilis, if the conditions mentioned above are not present.

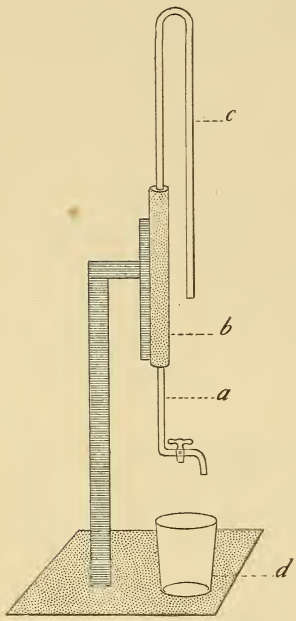

FIG. 37.—BLOOD. Siphon pipette (Barta). A broken burette, *a*, is attached to the glass tubing, *c*, by means of rubber tubing, *b*. The fluid to be siphoned is pushed up on *c* as it is drawn off into a tumbler, *d*.

Sera.—Blood is drawn from the vein at the bend of the elbow by means of a syringe and placed in sterile cork-stoppered test-tubes (120 × 13 mm.). The tube, with the brown slip wrapped about it, is placed on ice and the test made within forty-eight hours. Not less than 2 c.c. of blood should be collected and at times this

amount may be collected from the ear and in other ways. When the test is to be made, the tube and its brown slip are given a number. The serum is removed from the tube with a capillary pipette made from glass tubing provided with a nipple for suction (use a different pipette for each serum) and paced in the same size tube bearing the same number.

If the serum is not clear, then the clot in the original tube is thoroughly broken up with a sterile glass rod and the tube placed

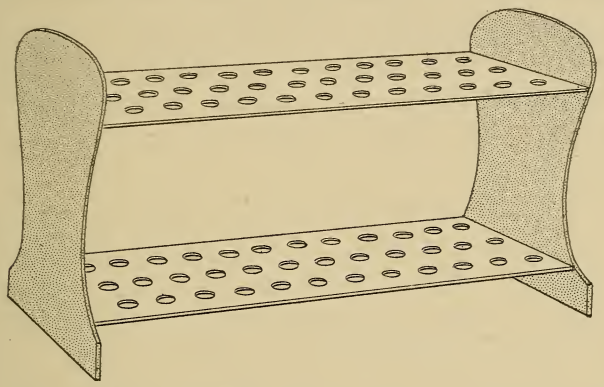

FIG. 38.—BLOOD. Rack for Wassermann tubes. 30 by 12 by 8 cm. made from thin galvanized iron. The ends are made in two pieces and crimped together at their center. This is not shown in the figure.

in the centrifuge and whirled five minutes when the clear serum may be pipetted off. Use separate pipettes and glass rods for each specimen. After using, the rods, pipettes and dirty tubes are placed in a wash basin and washed, after which they are dried and sterilized. The properly numbered tubes containing the clear serum are inactivated in a water bath at 55°C. for thirty minutes; 0.1 c.c. of clear serum is used for each test. A 1-c.c. pipette graduated in hundredths to the very tip is used for measuring the serum. Before measuring the second serum, the pipette is rinsed four times with sterile saline by drawing the solution into it and then blowing it out again. 1 c.c. of non-inactivated cerebro-spinal fluid per test is used. By placing 0.1 c.c. of the serum in drops on paper and letting dry, a "positive +++" serum may be kept for three months.

Complement.—If the number of reactions to be done is above thirty, it is perhaps better to obtain the blood by cutting the throats of guinea-pigs with a brain knife after a numbing knock on the head and allowing the blood to drip into a 10-cm. porcelain dish. A second person defibrinates by stirring with a heavy nickel-plated wire or a heavy platinum wire.

If there are fewer tests the blood is removed from the heart of the etherized guinea-pig strapped to a holder (Fig. 51). In this way several (8 c.c.) of blood may be removed from a large pig. A fine platinum-iridium needle should be used. The blood is at once forced from the syringe into a sterile 30-c.c. Erlenmeyer flask containing six pieces (1 cm. square) of bright fine-mesh wire gauze (Fig. 34). The flask is shaken by hand or in a mechanical defibrinator (Fig. 35).

After the blood is obtained in either of these ways and defibrinated, it is placed in sterile 15-c.c. centrifuge tubes and whirled for five minutes. Remove from the centrifuge and pipette off the clear serum. The same pipette that is used for measuring the serum may be used for measuring the complement. 0.05 c.c. is used for each test.

Amboceptor.—At intervals of three or four days (Tuesday and Friday of each week) inject 5, 8, 12, 15 and 20 c.c. of sterile, washed, sheep corpuscles into the peritoneal cavity of a rabbit weighing above 2000 gm.

Another good method for obtaining amboceptor is to give four injections of one to three cubic centimeters intravenously and bleed ten days after the last injection.

To obtain, the sterile, washed corpuscles, place 20 c.c. of defibrinated sheep's blood in a sterile 50-c.c. centrifuge tube and mark the upper surface of the blood with a blue pencil. Fill the tube with sterile saline and whirl for five minutes. Siphon off the supernatant fluid (Fig. 37), add saline and again centrifuge. Wash the corpuscle in this way four times. After decanting the saline the last time, add a sufficient amount of saline to bring the corpuscles to the blue mark.

Ten days after the injection of the last dose, take sufficient

blood from the ear to test the strength of the serum according to the directions given under "Titration for Amboceptor Unit." If satisfactory, fasten the animal in a rabbit holder, shave the neck, anesthetize, and dissect out both carotids for at least one inch. Tie one carotid as high as possible and place a spring artery clamp below. Cut just below the ligature, secure a firm hold in the adventitia of the loose end of the artery so as to guide the vessel into the mouth of the flask and then remove the clamp. The blood is collected in a sterile 250-c.c. Erlenmeyer flask.

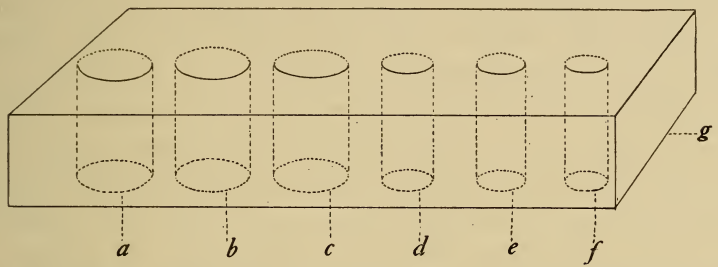

FIG. 39.—BLOOD. Block for amboceptor, etc. *a*, 50-c.c. centrifuge tub containing saline for washing pipettes; *b*, 50-c.c. centrifuge tube containing saline for making dilutions; *c*, 50-c.c. centrifuge tube containing 5 per cent. suspension of washed sheep's corpuscles; *d*, 155 by 16 mm. test-tube for antigen-complement—amboceptor-saline mixture of which 1 c.c. per test is used; *e*, properly diluted antigen; *f*, properly diluted amboceptor; *g*, block of wood 12 by 2½ by 1½ inches. Each tube is provided with its own pipette before beginning the tests.

Place on ice and allow the clot to harden over night. Remove and inactivate the serum. Seal 0.1 c.c. in small Wright pipettes (Fig. 32). Make the proper dilution (1–100 or 1–200) just before making the tests. Measure with the 1-c.c. pipette graduated in hundredths. For the test use two units.

To determine one unit, dilute the inactivated serum 1–100 or 1–200 and use the amounts indicated under "Titration for Amboceptor Unit." The smallest amount that completely hemolyzes all the corpuscles is one unit. It should be less than 0.07 c.c. of the 1–100 dilution. If the serum is not stronger than this at the time the test titration is made on the ear, it is preferable to immunize another animal.

Antigen.—The antigens used in the Wassermann test are not specific and more changes have been made in the antigens since

the test was first described than in any other part of the technic. At the present time the cholesterin antigen is said to give positive reactions in syphilitics when the acetone-insoluble antigen gives negative results. On the other the cholesterin antigen appears to give a greater number of positive reactions in non-syphilitics. Again no serum gives a positive reaction with acetone-insoluble antigen and a negative one with cholesterin.

For these reasons all the sera are tested with the cholesterin antigen first and if negative no further tests are required. The sera that are positive with cholesterin antigen are done with the acetone-insoluble antigen and the results with both antigens reported. The significance of "positive +," "positive ++," and "positive +++" given for the interpretation of results in the introduction refers to the acetone-insoluble antigen. The result with the acetone-insoluble antigen is the one that should be considered in the initial diagnosis of a case, while the result with the cholesterin antigen is of value in cases under treatment.

Cholesterin Antigen.—To 500 c.c. of absolute alcohol, add 2 gm. of cholesterin (Kahlbaum). Before making the test titrations, add 9 c.c. of saline to 1 c.c. of this alcoholic solution. After the titration make such a dilution that 0.1 c.c. of it is used per test. That is dilute so that 0.1 c.c. = twice the antigenic dose.

With the cholesterin antigen some use for each test four instead of two times the antigenic dose. This is possible, for the anticomplementary property of the cholesterin antigen is much less than that of the acetone-insoluble. The 1-c.c. pipette graduated in hundredths may be used. For the test use twice the antigenic dose.

Acetone-insoluble Antigen.—Place in a pint Mason jar 50 gm. fresh human or beef heart that has been ground in a sausage grinder and add 450 c.c. absolute alcohol. Place in the incubator at 37° for ten days shaking repeatedly. Filter through paper, place the filtrate in a flat dish 12 inches across and evaporate with an electric fan. Take up the residue in a dish with 200 c.c. of ether. Place the milky ether in a Mason jar over night. The following morning decant the ether which is now clear into a

beaker and evaporate to about 50 c.c. Then add 450 c.c. of acetone (C. P.) to the 50 c.c. remaining, thoroughly mix and decant the supernatant solution from the insoluble residue. After decanting, it is usually necessary to evaporate the acetone extract to the desired sticky mass with a fan. The sticky mass contains the antigenic lipoids. To 0.3 gm. add 1 c.c. ether and 9 c.c. of methyl alcohol.

To 1 c.c. of this ether-alcohol solution add 9 c.c. of saline and make the test titrations as outlined below. If the titration is satisfactory seal in tubes about 2 c.c. of the ether-alcohol solution per tube. Each time before starting to make Wassermann tests, such a dilution is made with saline that 0.1 c.c. of the resulting emulsion contains twice the antigenic dose. The stock antigen in the 2-c.c. tubes keeps for several years.

Gonococcus Antigen.—Grow the gonococci twenty-four hours on hydrocele or ascitic agar slants, take up the growth from each tube with 2 c.c. of distilled water by means of a bulb pipette (Fig. 36) and place the suspension of gonococci in a 500-c.c. glass-stoppered bottle. Shake the bottle in a mechanical shaker (Fig. 35) over night. Heat at 56°C. for two hours. Filter through a Berkefeld filter and add 0.9 per cent. sodium chloride and 0.5 per cent. phenol. Make such a dilution of this solution that 0.1 c.c. contains twice the antigenic dose. Some prefer to make gonococcus antigen from a number of different strains of the organism. Bacterial antigens other than gonococcus are prepared in the same way.

Testing the Antigens.—All antigens must be tested out before using. To make these initial tests, 9 c.c. of saline are added to 1 c.c. of the stock antigen. In order that 0.1 c.c. contain the amount that must be used for each test, variations from this 10 per cent. emulsion are later made according to the result of these titrations.

Hemolytic Property.—If an antigen is hemolytic in ten times the antigenic dose it should not be used. To test place 1 c.c. of the 10 per cent. emulsion, 1 c.c. of saline and 0.5 c.c. of 5 per cent. suspension of corpuscles in a test-tube and incubate one hour at

37.5°C. The liquid above the sedimenting corpuscles should not be tinged with red.

Anticomplementary Property.—Ten times the antigenic dose should not be anticomplementary (*i.e.*, it should not contain a substance that destroys the action of the complement and thus gives false positives). To test, place 1 c.c. 10 per cent. emulsion of antigen, 1 c.c. saline, 0.05 c.c. complement and two units amboceptor in a test-tube and incubate thirty-five minutes at 37.5°C. Then add 0.5 c.c. of 5 per cent. suspension of corpuscles and place in the incubator at 37.5°C. for one hour.

It is usually preferable, however, to determine the exact amount that is anticomplementary. To do this, instead of setting up a single tube as above, place eight tubes in a rack and add a decreasing amount of the antigen to each: 2, 1.5, 1, 0.8, 0.6, 0.4, 0.2, and 0.1 c.c. of the 10 per cent. emulsion. The other substances are added in the amounts indicated above in the case of the single tube where 1 c.c. of antigen is used. The tube containing ten times the antigenic dose as determined below should show complete hemolysis.

Antigenic Property.—To determine the antigenic property of a new antigen run the titration according to directions for the actual test (see p. 65), except that eight tubes are set up instead of one, and in these tubes there are placed decreasing amounts of the emulsion of antigen: 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05 and 0.02 c.c. The serum added to each tube is 0.1 c.c. of a known "positive ++++" serum.

Such dilutions of the stock antigens are made that 0.1 c.c. of these will be twice the smallest amount that gives complete inhibition of hemolysis in the test of the antigenic property. Ten times this proper antigenic dose should not be hemolytic and should not be anticomplementary.

In the case of cholesterin antigen, there is no difficulty in obtaining an antigen that fulfills these requirements, but it is much more difficult to secure an acetone-insoluble antigen that is not anticomplementary in ten times the antigenic dose. In the case of bacterial antigens it is even more difficult, and in some

cases it may become necessary to use an antigen that becomes anticomplementary at three times the antigenic dose. This is the very shortest "working distance" that is permissible.

Corpuscles.—Collect the blood from slaughter-house lambs in a sterile pint Mason jar containing glass beads. Collect half full and then shake until completely defibrinated (ten minutes). Keep on ice until used. With a 10-c.c. pipette transfer 10 c.c. of the defibrinated blood to a 50-c.c. centrifuge tube and mark the upper limit of the blood with a blue pencil. Make up to the 50-c.c. mark with the saline and then centrifuge 5 minutes. Repeat this three times. After the last centrifugation make up to the original 10 c.c. (blue mark) and to these 10 c.c. add 190 c.c. of saline in a 250-c.c. Erlenmeyer flask. Keep on ice. Use a 10-c.c. pipette graduated in tenths for measuring out the corpuscles. For the test use 0.5 c.c. of the 5 per cent. suspension of corpuscles.

For a limited number of Wassermann tests, it is often preferable to remove the blood from the jugular vein of a sheep kept for this purpose. One person should straddle the sheep holding its chin up while the other clips off the wool and inserts into the vein a small platinum-iridium needle attached to a 20-c.c. syringe. Defibrinate the blood in a 250-c.c. flask (Fig. 34) containing disks of wire gauze. Wash in 15-c.c. centrifuge tubes. A siphon pipette (Fig. 37) is very convenient for removing the supernatant fluid while washing. Never vary the technic of making up the corpuscle suspension.

Saline.—Add 8.8 gm. sodium chloride (Merck U. S. P.) to a liter of distilled water. Autoclave. For use place the saline in 50-c.c. centrifuge tubes supported in a block (Fig. 39) in which there are placed 10-c.c. pipettes graduated in tenths. There should be two of these; one is used for washing the pipette used for measuring the sera and the other for diluting the ingredients to the correct amounts.

TEST.—Minor modifications of technic are very numerous. However, the general principles of the test are the same in all laboratories where the most pains-taking care is used. In a test

where the technic is so complicated, there is no excuse for introducing a factor which will certainly increase the chance of error.

In the test, six liquids are placed together in a single test-tube: patient's serum (0.1 c.c.), complement (0.05 c.c.), amboceptor (two units), antigen (0.1 c.c. = twice the antigenic dose), corpuscles (0.5 c.c.), saline (q.s. 1.6 c.c.). The patient's serum and the corpuscles are added separately but the use of 1 c.c. containing the other four (an antigen-complement-amboceptor-saline mixture) reduces the number of measurements for each test to three.

Before beginning the test (this may be done while the "Titration for amboceptor unit" is being made), fill tube *e*, Fig. 39, with antigen. Suppose in the titration of the antigen for antigenic property it is found that 0.04 c.c. of a trial 10 per cent. emulsion is the antigenic dose. Then 0.08 c.c. of a 10 per cent. emulsion or 0.1 c.c. of 8 per cent. emulsion is twice the antigenic dose. In this case in order that 0.1 c.c. may contain twice the antigenic dose dilute the stock antigen 8–100. The dilution that is to be made is marked on each stock antigen.

As soon as the "Titration for amboceptor unit" is complete, fill tube *d*, Fig. 39. This is done according to "antigen-complement-amboceptor-saline table."

The sera to be tested are inactivated, numbered consecutively and placed in the front row of a rack (Fig. 38).

The 5 per cent. suspension of corpuscles is in *c*, Fig. 39. The three liquids then to be used in the actual test are in the test-tube rack, Fig. 38, and in *c* and *d*, Fig. 39.

Titration for Amboceptor Unit.—Place eight test-tubes (120 × 13 mm.) in the front row of a rack (Fig. 38) and to each of these add the amounts of diluted amboceptor (Fig. 39) indicated below. Add to each tube 0.05 c.c. 5 per cent. suspension sheep corpuscles and 1.0 c.c. saline.

Incubate in the water-bath thirty-five minutes at 37.5°C. The last tube in which hemolysis is complete is one unit. This should be between 0.02 and 0.07 c.c.

Amboceptor diluted	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
Complement undiluted G. P. serum	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sheep corp. 5 per cent. susp	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Normal saline	1.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Technic for the Actual Test.—The patients' sera, the corpuscles and the antigen-complement-amboceptor-saline mixture are now ready for the test. The cholesterin antigen-complement-amboceptor-saline mixture is contained in *d*, Fig. 39, and, inasmuch as there are usually positive cases even where a small number of tests are made, a second tube directly behind *d* should contain acetone-insoluble "a-c-a-s" mixture. If a gonococcus complement fixation test is to be done then another tube placed in the rear of the block must contain gonococcus "a-c-a-s" mixture.

1. For each Wassermann test to be done place a tube (120 × 13 mm.) in the front row of a rack (Fig. 38). In next to the last hole of the series place a tube for a known positive serum and in the last hole a tube for a known negative serum.

If a gonococcus test is to be done place a tube in the second row directly back of the one in the front row.

2. Add to each tube 0.1 c.c. of patient's serum (inactivated thirty minutes at 54° to 55°). Use for measuring, a pipette graduated in hundredths to the very tip. Keep this pipette in *a*, Fig. 39, and wash four times between each serum by drawing it three-fourths full of saline and then blowing out into the sink.

3. To each tube add 1 c.c. of cholesterin "a-c-a-s" mixture. If there is a gonococcus tube in the second row add, of course, 1 c.c. of gonococcus "a-c-a-s" mixture to it.

4. Incubate in water-bath thirty-five minutes at 37.5°C.

5. Add 0.5 c.c. of 5 per cent. sheep corpuscles to each tube.

6. Incubate in water-bath one hour at 37.5°C.

7. Read results.

8. For each positive Wassermann place a tube properly

Antigen-complement-amboceptor-saline Table

Use 1 C.c. of this Mixture per Test

No. of tests	Antigen 0.1 c.c. of proper dilution	Complement 0.05 c.c. undiluted	Amboceptor, 2 units	Sterile normal saline, q.s. ad 1 c.c.
5	0.5 c.c.	0.25 c.c.	0.04–0.2 c.c. 0.06–0.3 c.c. 0.08–0.4 c.c. 0.1 –0.5 c.c. 0.12–0.6 c.c. 0.14–0.7 c.c.	4.05 c.c. 3.95 c.c. 3.85 c.c. 3.75 c.c. 3.65 c.c. 3.55 c.c.
10	1.0 c.c.	0.5 c.c.	0.04–0.4 c.c. 0.06–0.6 c.c. 0.08–0.8 c.c. 0.1 –1.0 c.c. 0.12–1.2 c.c. 0.14–1.4 c.c.	8.1 c.c. 7.9 c.c. 7.7 c.c. 7.5 c.c. 7.3 c.c. 7.1 c.c.
15	1.5 c.c.	0.75 c.c.	0.04–0.6 c.c. 0.06–0.9 c.c. 0.08–1.2 c.c. 0.1 –1.5 c.c. 0.12–1.8 c.c. 0.14–2.1 c.c.	12.15 c.c. 11.85 c.c. 11.55 c.c. 11.25 c.c. 10.95 c.c. 10.65 c.c.
20	2.0 c.c.	1.0 c.c.	0.04–0.8 c.c. 0.06–1.2 c.c. 0.08–1.6 c.c. 0.1 –2.0 c.c. 0.12–2.4 c.c. 0.14–2.8 c.c.	16.2 c.c. 15.8 c.c. 15.4 c.c. 15.0 c.c. 14.6 c.c. 14.2 c.c.
25	2.5 c.c.	1.25 c.c.	0.04–1.0 c.c. 0.06–1.5 c.c. 0.08–2.0 c.c. 0.1 –2.5 c.c. 0.12–3.0 c.c. 0.14–3.5 c.c.	20.25 c.c. 19.75 c.c. 19.25 c.c. 18.75 c.c. 18.25 c.c. 17.75 c.c.
30	3.0 c.c.	1.5 c.c.	0.04–1.2 c.c. 0.06–1.8 c.c. 0.08–2.4 c.c. 0.1 –3.0 c.c. 0.12–3.6 c.c. 0.14–4.2 c.c.	24.3 c.c. 23.7 c.c. 23.1 c.c. 22.5 c.c. 21.9 c.c. 21.3 c.c.
35	3.5 c.c.	1.75 c.c.	0.04–1.4 c.c. 0.06–2.1 c.c. 0.08–2.8 c.c. 0.1 –3.5 c.c. 0.12–4.2 c.c. 0.14–4.9 c.c.	28.35 c.c. 27.65 c.c. 26.95 c.c. 26.25 c.c. 25.55 c.c. 24.85 c.c.
40	4.0 c.c.	2.0 c.c.	0.04–1.6 c.c. 0.06–2.4 c.c. 0.08–3.2 c.c. 0.1 –4.0 c.c. 0.12–4.8 c.c. 0.14–5.6 c.c.	32.4 c.c. 31.6 c.c. 30.8 c.c. 30.0 c.c. 29.2 c.c. 28.4 c.c.
45	4.5 c.c.	2.25 c.c.	0.04–1.8 c.c. 0.06–2.7 c.c. 0.08–3.6 c.c. 0.1 –4.5 c.c. 0.12–5.4 c.c. 0.14–6.3 c.c.	36.45 c.c. 35.55 c.c. 34.65 c.c. 33.75 c.c. 32.85 c.c. 31.95 c.c.

ANTIGEN-COMPLEMENT-AMBOCEPTOR-SALINE TABLE.—*Continued*

Use 1 C.c. of this Mixture per Test

No. of tests	Antigen 0.1 c.c. of proper dilution	Complement 0.05 c.c. undiluted	Amboceptor, 2 units	Sterile normal saline, q.s. ad 1 c.c.
50	5.0 c.c.	2.5 c.c.	0.04–2.0 c.c. 0.06–3.0 c.c. 0.08–4.0 c.c. 0.1 –5.0 c.c. 0.12–6.0 c.c. 0.14–7.0 c.c.	40.5 c.c. 39.5 c.c. 38.5 c.c. 37.5 c.c. 36.5 c.c. 35.5 c.c.
55	5.5 c.c.	2.75 c.c.	0.04–2.2 c.c. 0.06–3.3 c.c. 0.08–4.4 c.c. 0.1 –5.5 c.c. 0.12–6.6 c.c. 0.14–7.7 c.c.	44.55 c.c. 43.45 c.c. 42.35 c.c. 41.25 c.c. 40.15 c.c. 39.05 c.c.
60	6.0 c.c.	3.0 c.c.	0.04–2.4 c.c. 0.06–3.6 c.c. 0.08–4.8 c.c. 0.1 –6.0 c.c. 0.12–7.2 c.c. 0.14–8.4 c.c.	48.6 c.c. 47.4 c.c. 46.2 c.c. 45.0 c.c. 43.8 c.c. 42.6 c.c.
65	6.5 c.c.	3.25 c.c.	0.04–2.6 c.c. 0.06–3.9 c.c. 0.08–5.2 c.c. 0.1 –6.5 c.c. 0.12–7.8 c.c. 0.14–9.1 c.c.	52.65 c.c. 51.35 c.c. 50.05 c.c. 48.75 c.c. 47.45 c.c. 40.15 c.c.
70	7.0 c.c.	3.5 c.c.	0.04–2.8 c.c. 0.06–4.2 c.c. 0.08–5.6 c.c. 0.1 –7.0 c.c. 0.12–8.4 c.c. 0.14–9.8 c.c.	56.7 c.c. 55.3 c.c. 53.9 c.c. 52.5 c.c. 51.1 c.c. 49.7 c.c.
75	7.5 c.c.	3.75 c.c.	0.04 –3.0 c.c. 0.06 –4.5 c.c. 0.08 –6.0 c.c. 0.1 –7.5 c.c. 0.12 –9.0 c.c. 0.14–10.5 c.c.	60.75 c.c. 59.25 c.c. 57.75 c.c. 56.25 c.c. 54.75 c.c. 53.25 c.c.
80	8.0 c.c.	4.0 c.c.	0.04 – 3.2 c.c. 0.06 – 4.8 c.c. 0.08 – 6.4 c.c. 0.1 – 8.0 c.c. 0.12 – 9.6 c.c. 0.14 –11.2 c.c.	64.8 c.c. 63.2 c.c. 61.6 c.c. 60.0 c.c. 58.4 c.c. 56.8 c.c.
85	8.5 c.c.	4.25 c.c.	0.04 – 3.4 c.c. 0.06 – 5.1 c.c. 0.08 – 6.8 c.c. 0.1 – 8.5 c.c. 0.12 –10.2 c.c. 0.14 –11.9 c.c.	68.85 c.c. 67.15 c.c. 65.45 c.c. 63.75 c.c. 62.50 c.c. 60.35 c.c.
90	9.0 c.c.	4.5 c.c.	0.04 – 3.6 c.c. 0.06 – 5.4 c.c. 0.08 – 7.2 c.c. 0.1 – 9.0 c.c. 0.12 –10.8 c.c. 0.14 –12.6 c.c.	72.9 c.c. 71.1 c.c. 69.3 c.c. 67.5 c.c. 65.7 c.c. 63.9 c.c.

FIG. 40.—BACTERIOLOGICAL SPECIMENS. Sterile cotton swabs. Dry-heat sterilize and then autoclave. Use one swab for making culture and the other for making smear on a slide. *a*, Wooden applicators obtained in boxes; *b*, small amount of cotton firmly wrapped by rotating *a*.

numbered with blue pencil in the front row of a rack and directly behind it a second tube. The tubes in the front row are for acetone-insoluble antigen and those in the second row are for controls. Therefore with the tubes in the front row carry out the steps 1–7 inclusive using acetone-insoluble "a-c-a-s" mixture instead of the cholesterin. With the tubes in the second row carry out the steps 1–7 inclusive but use "c-a-s" mixture (a mixture identical with the "a-c-a-s" mixture except that it contains no antigen). No antigen is added to the control tubes in the second row. All second row tubes should show complete hemolysis. If they do not the test must be reported "anti-complementary."

Even when only a single test is to be performed it is better to make up the "a-c-a-s" mixture as indicated. But in such a case the test with the acetone-insoluble antigen may be run in the third and the control in the second row, so that all three are run at the same time. In laboratories, however, where there are large numbers of tests to be performed it is a useless waste of complement to run controls on negative sera.

BACTERIOLOGICAL SPECIMENS

Introduction.—Material for cultures is most commonly sent to the laboratory on cotton swabs (Fig. 40). If the pus or other material is placed in a test-tube and presented for cultural purposes, the cultures may be made with a sterile platinum loop or if fluid with a sterile pipette.

Whether the material to be examined bacteriologically is sent on a swab, in a test-tube, or otherwise, it must be accompanied by a brown slip (Fig. 41) filled out by the clinician.

REPORT FOR THE PATHOLOGIST

Date.....

Name.....

Sex.....Age.....

.....Service, Ward.....Bed.....

If not a hospital patient, give name and address of physician.....

.....

Duration and Nature of Disease.....

.....

.....

.....

Place from which each piece of tissue for diagnosis is removed.....

.....

Clinical Diagnosis.....

Diagnosis of Pathologist.....

Signed,

.....Pathologist

Instruction to House Officers

All tissue must be immediately wrapped in sterile gauze, moistened with salt solution and then wrapped with oiled paper and the brown slip.

FIG. 41.—BACTERIOLOGICAL SPECIMENS. Brown slip. This slip of light brown paper 6½ by 9½ inches must be filled out by the clinician and sent to the laboratory with all surgical and bacteriological specimens. As soon as a diagnosis is made it is written on this slip which is at once returned to the clinician.

A number of systems for numbering and recording specimens are in use. Perhaps the simplest one is to indicate bacteriological specimens by B, surgical specimens by S, and autopsy specimens by A, and to place the last two numerals of the current year after these letters to show in what year the specimen was received. Following this system the first bacteriological specimen received in 1916 bears the number B 16.1 and the 515th specimen bears

B. Diagnosis:

Material:

Name: Service:

Clinical Diagnosis:

Smear (Stain): Date { Received / Reported

Agar:

Litmus Milk:

FIG. 42.—BACTERIOLOGICAL SPECIMENS. Index card for bacteriological records. Cards 5 by 7 inches. All cards for indexing should be this size.

the number B 16.515, etc. At the time the brown slip is removed from about the specimen the bacteriological number is written on the bottom of the slip. The consecutive numbers are kept on a sheet and as each specimen is given a number, this one is crossed off on that sheet. The number is also placed on a card (Fig. 42) for the laboratory record.

As a routine two swabs are sent to the laboratory. With one

of these a smear is made on a slide and stained by Gram's method. With the other a culture is made first on an agar slant by gently rubbing it over the agar surface and then in a tube of litmus milk. Other media are used if indicated.

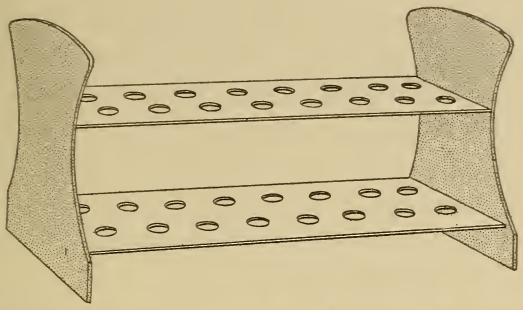

FIG. 43.—BACTERIOLOGICAL SPECIMENS. Rack for bacteriological cultures Wooden test-tube rack for sixteen culture tubes. In racking up a number of separate cultures, place agar-slant cultures in front row and the litmus-milk cultures in back row.

The bacteriological number (Example: B 16.515 or just 515) is written on each inoculated tube with a pencil. The cultures are placed in a rack (Fig. 43) with the agar cultures in the front row. The rack is placed in the incubator at 37°. The following morning a smear is made from each of the agar slants and each milk culture and stained by Gram's method (p. 79). In examining colonies from a plate or a large number of tube or flask cultures it is very convenient to make about eight preparations on a slide (Fig. 44), marking the exact position of each with a blue pencil at the margin of the slide. Place preparation No. 1 at the left end of the slide.

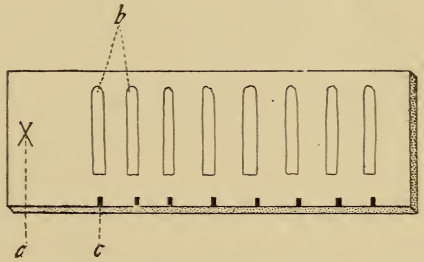

FIG. 44.—BACTERIOLOGICAL SPECIMENS. Slide with eight preparations. *a*, Cross made with a wax pencil. The preparation next to this cross is number one. *b*, Eight small drops of water placed on the slide with pipette, Fig. 56. Add the material from the cultures with platinum loop and spread each out as indicated. Mark the exact position of each preparation on the margin *c* with a wax pencil.

The cards (Fig. 42) are kept during the current year in a

card index case in the order of the numbers on them. The cards for previous years may be tied together and filed away. There must be a card for each number. As soon as ascertained, the diagnosis is written on the brown slip which is signed and returned to the clinician.

Sterile swabs (Fig. 40) are best prepared by wrapping one end of the wooden throat applicator firmly with cotton, placing two of these in a test-tube and plugging the upper end of the tube with cotton. The tubes are dry-heat sterilized and then autoclaved. The lower ends of applicators should reach the bottom of the tubes and the upper ends should project one inch or more. One of the swabs is used for making direct smears on slides and the second is used for making cultures.

For routine diagnosis, the culture media and the stains should be made as simple as possible. The details of the preparation and the application of the media and stains used should be thoroughly understood.

Media.—The number of bacteria present may be so small that they cannot be found in smears of the material; it may be impossible to identify organisms from smears alone; or it may be desirable to test the biologic characters of the organism. In such cases the organisms must be grown on media in cultures and often in pure cultures. It is also necessary to grow the organisms on artificial media for the preparation of vaccines, antigens, etc.

Plating.—For the isolation of bacteria in pure cultures, the individual bacteria are mechanically separated from each other to a distance of at least 1 cm. This may be done by mechanically rubbing the bacteria-containing substance over the surface of a solid medium, or more often by mixing it with melted agar (agar melted at 100° and cooled to 45°) that becomes solid on cooling. The melted agar after inoculation is usually poured into a Petri dish so as to get a flat surface. The single separate bacterial cells multiply in the medium sufficiently to give rise to a macroscopic mass, a colony. Such a colony of course consists of a single kind of organism as it comes from a single bacterial cell.

Tube Cultures.—For obtaining cultures that are not necessarily

pure and for making subcultures from colonies and other pure cultures, media in tubes are usually employed.

Agar.—Dissolve 5 gm. Liebig's meat extract, 5 gm. salt and 10 gm. peptone in 1000 c.c. of water at 55°C. Titrate 0.8 per cent. acid.

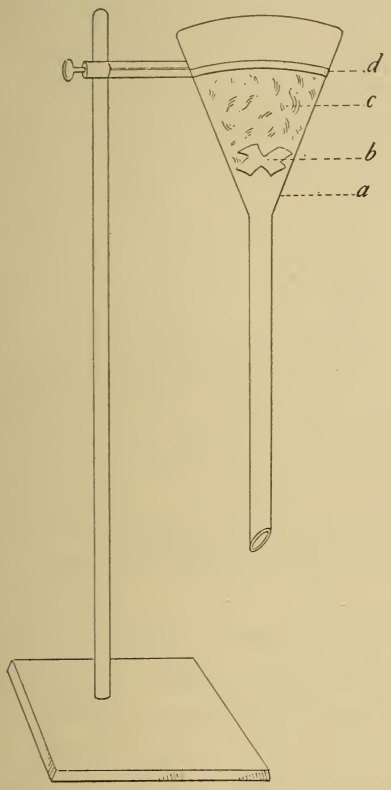

FIG. 45.—BACTERIOLOGICAL SPECIMENS. Funnel for filtering media. *a*, 20-cm. funnel; *b*, large cork notched with knife; *c*, thin layer of cotton; *d*, iron ring supporting funnel.

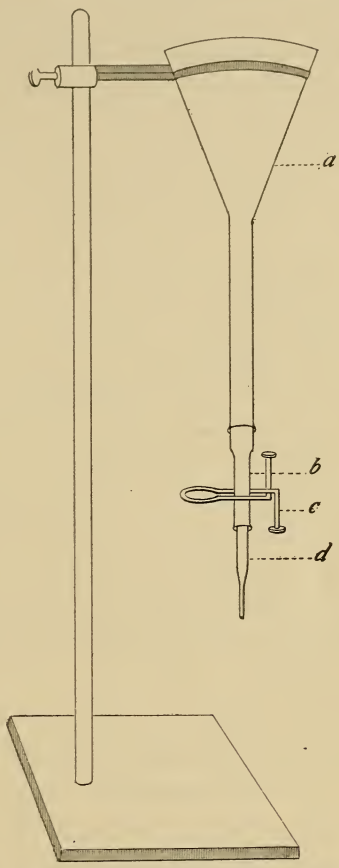

FIG. 46.—BACTERIOLOGICAL SPECIMENS. Funnel for tubing media. *a*, 15-cm. funnel; *b*, soft rubber tubing; *c*, pinch cock; *d*, glass tip.

The titration is made on 5 c.c., removed with a graduated pipette and placed in a small porcelain dish. Add 20 c.c. of distilled water and boil for one minute. While hot add 2 drops of 0.5 per cent. alcoholic solution of phenolphthalein and run in

twentieth-normal sodium hydroxide until the first distinct pink appears. The number of cubic centimeters of sodium hydrate used multiplied by 10 gives the number of cubic centimeters of normal alkali required to neutralize 1 liter. Suppose 1.5 c.c. of the twentieth-normal hydrate are used for the 5 c.c. then $1.5 \times 10 = 15$ c.c. of N. alkali required to neutralize 1 liter. With this titration, to make the liter 0.8 per cent. acid (0.8 per cent. of 1000 = 8), add 7 c.c. (15 c.c. − 8 c.c. = 7 c.c.) of normal hydroxide.

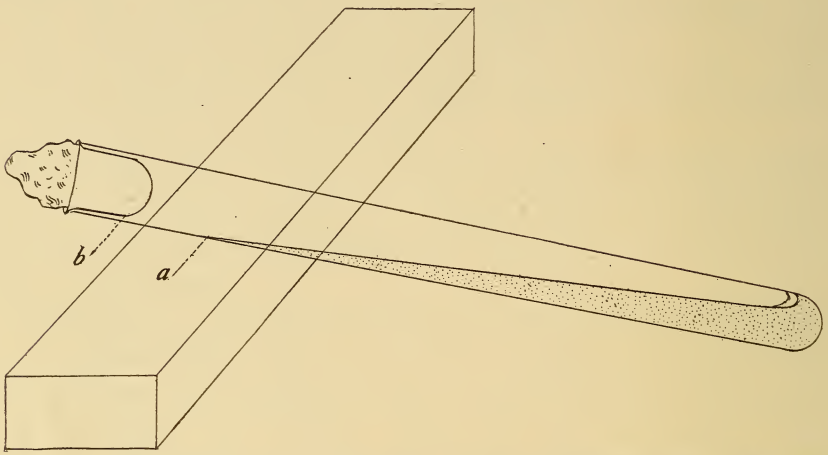

FIG. 47.—BACTERIOLOGICAL SPECIMENS. Slants of solid media. The upper portion of the slant *a* should reach to within 1 inch of the lowermost part of the cotton plug *b*.

After adjusting the reaction add 30 gm. of pulverized agar or 20 gm. unpowdered agar, boil ten minutes, make up to 1000 c.c., cool the medium to 45°, and thoroughly stir in the white of one egg. Raise slowly to the boiling point and boil gently for five minutes without stirring or agitating the mixture. The egg coagulates in large masses and the clear liquid readily runs through muslin placed in a large funnel provided with a cork at its opening. A thin layer of cotton is placed over the muslin. The hot agar is poured on only so fast as it filters through (Fig. 45).

For slants, fill the tubes from the funnel used for tubing (Fig. 46) to such a height that the upper edge of the slant lacks 1 inch of

reaching the cotton plug, while the bottom of the tube is not completely covered with medium (Fig. 47).

To make *glycerine agar* from the plain agar, add 2 per cent. of twice distilled glycerine to the desired quantity of melted agar in a flask, mix and tube. For *glucose agar* add 2 per cent. glucose.

Arnoldize all sugar media and gelatin and autoclave all other simple media at 110°C. for twenty minutes. All glassware cleaned and plugged with cotton or placed in containers must be heated in the dry-heat oven at 170° to 200°C. for fifteen minutes.

To prepare *blood agar* from the plain sterile slants, add about 20 per cent. of sterile defibrinated rabbit or human blood from a pipette to the agar tubes melted and cooled to 45°C. Thoroughly mix by rolling. Slant. The sterile non-defibrinated blood from the arm vein of a patient may be added directly from the syringe. To prepare *hydrocele or ascitic agar*, add these fluids in the same way that the blood was added. All slants should be placed in the incubator for twenty-four hours to test their sterility and they should be kept permanently in the slanted position until used. The preparation of these media differs from the preparation of glycerine and sugar media in that the material added must be kept sterile. To do this a sterile bulb pipette (Fig. 36) is very useful but sterile 10 c.c. pipettes (*h*, Fig. 33) may be used.

Bouillon.—Dissolve 5 gm. Liebig's meat extract, 5 gm. salt and 10 gm. peptone in 1000 c.c. water. Boil fifteen minutes, make up to one liter, titrate to 0.8 per cent. acid, autoclave at 110° for twenty minutes, and filter through filter paper. Place in flasks or tubes and autoclave at 110°C. for twenty minutes.

For the cultivation of some bacteria, it is desirable to obtain the meat extractives directly from the meat. In cases where the laboratory facilities are good it is advisable to use chopped beef as a routine. To use the beef, "1000 c.c. of beef infusion" is substituted in the directions for "5 gm. Liebig's meat extract" and the liter of water omitted.

The "beef infusion" is prepared by adding 1000 c.c. of water to 500 gm. chopped, spice-free lean beef (beef ground in a sausage grinder), placing in the ice-box for twenty-four hours and then

filtering through muslin. The filtrate is the "beef infusion." Another way of preparing the "beef infusion" is to heat at 55°C. for one hour instead of allowing to stand for twenty-four hours in the ice-box. At times heat may remove desirable substances from the infusion. This may be avoided by adding the peptone and salt to the infusion, correcting the reaction and then passing through a Berkefeld filter (Fig. 48).

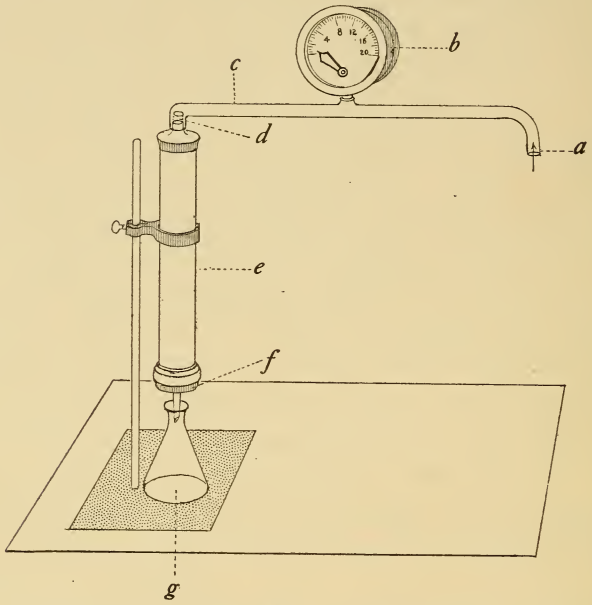

FIG. 48.—BACTERIOLOGICAL SPECIMENS. Berkefeld filter. *a*, Connects with pipe from compressed air apparatus; *b*, ordinary steam gage; *c*, heavy metal or rubber pipe. If *c* is rubber tubing then a small connection *d* must be screwed into the upper end of the metal case *e*. The bougie is attached to the milled end *f*. The filters are obtained from Aug. Giese and Son, 4 Cedar St., New York City. *g* is a sterile flask for reception of the filtrate. An excellent copper case (*e*) for pressure filtration may be obtained from Isaac Young and Co., Coppersmiths, East Boston. Suction may be used for filtration.

Litmus Milk.—To 1 liter of "fat-free" sweet milk, add sufficient 1 per cent. azolitmin to give the desired blue.

Gelatin.—To 1000 c.c. water add 5 gm. Liebig's beef-extract, 5 gm. salt and 10 gm. peptone and dissolve at 55°C. Make the gelatin neutral to phenolphthalein (see titration of agar); cool to 30°C. and thoroughly stir in the white of one egg; boil for five

minutes without stirring; filter through muslin and a thin layer of cotton. Tube.

Dunham's Solution.—To 1000 c.c. water add 10 gm. peptone and 5 gm. salt. Dissolve with heat and filter through paper.

Lactose Bile.—Autoclave one liter fresh ox-bile, filter, add two per cent. lactose, place in tubes and flasks and fractional sterilize.

Serum Water.—To 250 c.c. calf or pig serum, add 750 c.c. water. Add 1 per cent. azolitmin to pale blue color and 1 per cent. dextrose, mannose, lactose, saccharose, or inulin. Of these sugars the first two are monosaccharids (aldehyde-alcohols with six carbons), lactose and saccharose are disaccharids (former d glucose + d galactose and while the latter is made up of two d glucose molecules). Inulin is a polysaccharid. The approximate way (there are variations within the groups) in which organisms attack these sugars is shown in the attached table.

	Inulin	Saccharose	Lactose	Mannose	Dextrose
B. Coli	o	Few strains	Gas	Gas	Gas
B. Mucosus capsulatus	o	o	Acid	Gas	Gas
B. enteritidis	o	o	o	Gas	Gas
B. Paratyphosus B	o	o	o	Gas	Gas
B. Paratyphosus A	o	o	o	Gas	Gas
B. Typhosus	o	o	o	Acid	Acid
B. Dysenteriæ (Flexner)	o	o	o	Acid	Acid
B. Dysenteriæ (Shiga)	o	o	o	o	Acid
Micrococcus meningitidis	o	o	o	o	o
Strep. pyogenes	o	Acid	Acid	Acid	Acid
Strep. of subacute endocarditis	o	Acid	Acid	Acid	Acid
Strep. mucosus cap	Acid	Acid	Acid	Acid	Acid
Strep. pneumoniæ	Acid	Acid	Acid	Acid	Acid
Staph. pyogenes aureus	Acid	Acid	Acid	Acid	Acid

Loeffler's Blood Serum.—To 250 c.c. 1 per cent. glucose bouillon, add 750 c.c. calf or pig serum. Tube, slant, place in dry-heat oven or in an inspissator and very slowly raise the temperature to 85°C. and maintain that temperature for two hours. Autoclave.

Drigalski Medium.—Make 1 liter of agar in the usual way

except 10 gm. of nutrose are added and it is made neutral to phenolphthalein. To the melted agar add 1 per cent. of lactose, 1 per cent. of a 0.1 per cent. solution of crystal violet and enough 1 per cent. solution of azolitmin to give the desired color. Autoclave.

Petroff Medium for Isolation of Tubercle Bacilli.—To the material (sputum) in a centrifuge tube of suitable size (50 c.c.), add an equal amount of normal sodium hydroxide, stir thoroughly and place in the incubator at 37°C. for thirty minutes. Add normal hydrochloric acid to neutralize exactly the normal alkali used. Centrifuge and make smears or cultures from the sediment.

To prepare the medium, place eggs in alcohol for one-half hour, break into a sterile beaker and thoroughly beat. The beaten egg

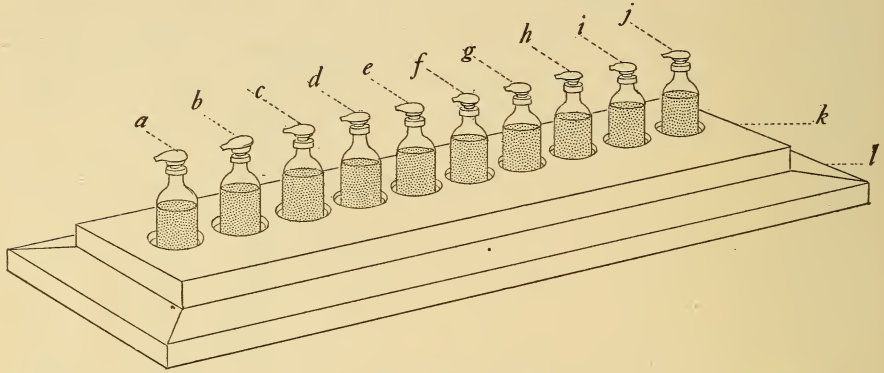

FIG. 49.—BACTERIOLOGICAL SPECIMENS. Block for stains. Bottles hold 30 c.c. and are obtained from Bausch and Lomb Optical Co. The block consists of a lower ¾-inch board 25 by 3.5 inches *k* on which there is tacked a board, *l*, that has in it ten 1.5-inch holes for the bottles. *a*, Gram's staining solution; *b*, Gram's iodine; *c*, 95 per cent. alcohol; *d*, pyronin; *e*, Ziehl-Neelsen; *f*, Czaplewsky; *g*, Loeffler's methylene blue; *h*, distilled water; *i*, polychrome stain for protozoa; *j*, polychrome blood stain.

is then measured in a sterile graduate. To 2 parts of the sterile egg, add 1 part of sterile glycerinated beef juice.

To prepare the sterile beef-juice, add 500 c.c. 15 per cent. glycerine to 500 gm. chopped beef and allow to stand over night. Filter through gauze, add 15 c.c. of 1 per cent. alcoholic solution of gentian violet and pass through a Berkefeld filter (N) into a sterile flask. After mixing the egg and beef-juice in the sterile flask, pour into a sterile funnel tubing apparatus and tube into sterile test-tubes. Place the sterile egg-beef-juice mixture in

tubes, slowly raise the temperature to 85°C. and hold it at that temperature for two hours. Autoclave.

Stains.—The number of stains used should be reduced to a minimum and the worker should familiarize himself with every detail of these. A ten-hole block (Fig. 49) for ten 30 c.c. staining bottles with snout-leaks is very convenient: (1) Gram's Staining Solution, (2) Gram's Iodine, (3) Pyronin, (4) Loeffler's Methylene Blue, (5) Ziehl-Neelsen, (6) Czaplewsky, (7) 95 per cent. Alcohol, (8) Blood Stain, (9) Polychrome Stain and (10) Distilled Water.

Write the labels and when dry apply thin celloidin with a small brush. As soon as this is dry paint over with balsam damar in xylol. The balsam damar in xylol is obtained from Bausch and Lomb.

Unless otherwise directed all bacterial preparations are fixed in the flame. Proper fixation must be acquired by experience. To fix a smear on a slide, hold the slide for ten seconds with the preparation side up 3 inches above a Bunsen burner flame 4 inches high. If the preparation is on a cover, hold the cover-glass an inch or two farther above the top of the flame for the same length of time.

Loeffler's Methylene Blue.—To 1485 c.c. of distilled water, add 500 c.c. of a saturated absolute alcoholic solution of methylene blue (to saturate place a bottle containing 2000 c.c. absolute alcohol and 30 gm. methylene blue in the paraffin oven for several hours and shake repeatedly) and 15 c.c. of 1 per cent. aqueous solution of potassium hydroxide. It stains in a few seconds and does not readily overstain. This stain brings out as much detail in the morphology of Klebs-Loeffler bacillus as any of the special stains.

Gram's Stain.—Two stock solutions are required: Solution *A* is made up by adding an excess of methyl violet to 330 c.c. absolute alcohol and 90 c.c. aniline oil in a 500-c.c. glass-stoppered bottle. Solution *B* is made by adding an excess of methyl violet to 400 c.c. of distilled water in a 500-c.c. glass-stoppered bottle.

To make the staining solution (Gram's staining solution), add 2 c.c. of *A* to 18 c.c. of *B* in a 30-c.c. staining bottle provided with

a snout-leak. The Gram's staining solution keeps two weeks. For this reason it is necessary to make the solution up fresh from the two stock solutions once in two weeks. Solutions *A* and *B* are permanent.

The fixed preparation is covered with the Gram's staining solution for three minutes. Wash off with water and apply Gram's iodine (iodine 1 gm., potassium iodide 2 gm. and water 300 c.c.) for thirty seconds. Wash the iodine off with water and then run 95 per cent. alcohol over the preparation for thirty seconds. Wash with water and stain with pyronin (pyronin 2 gm., 10 per

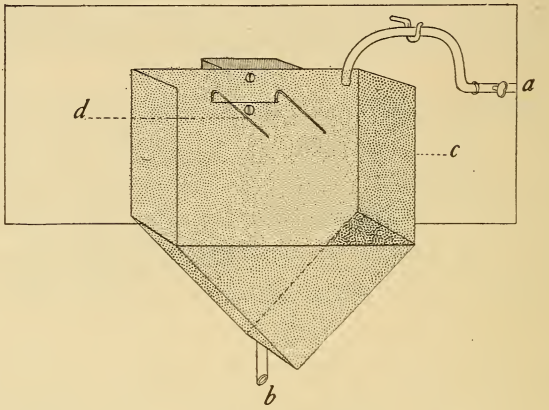

FIG. 50.—BACTERIOLOGICAL SPECIMENS. Support for tubercle bacillus stains (Barta). *a*, Rubber tubing provided with a cock and leading to an elevated water bottle; *b*, rubber tubing leading into sink; *c*, box of thin galvanized iron nailed against the wall; *d*, wire on which the slide is placed; the slide is heated with a Bunsen flame by occasionally waving the flame back and forth beneath it.

cent. formalin 10 c.c. and water 90 c.c.) for one minute. Wash, air dry and examine with the oil. Each individual must test the time for each step in the stain by using slides that have on them both Gram negative and Gram positive bacteria.

Ziehl-Neelsen Stain for Tubercle Bacilli.—To 450 c.c. of 5 per cent. carbolic acid in a glass-stoppered bottle, add 50 c.c. of a saturated absolute alcohol solution of fuchsin (neutral). To saturate the absolute alcohol with fuchsin place in tightly stoppered bottle in paraffin oven and shake. A stock bottle of the saturated fuchsin solution should be kept on hand.

Czaplewsky's solution for decolorizing the tubercle bacilli stains is made by adding 5 c.c. of concentrated hydrochloric acid, 5 gm. of sodium chloride and 200 c.c. of water to 1000 c.c. of 95 per cent. alcohol.

The smear properly fixed on a slide is placed on a staining support (Fig. 50), the Ziehl-Neelsen solution placed on the preparation and heated by occasionally waving a Bunsen flame beneath

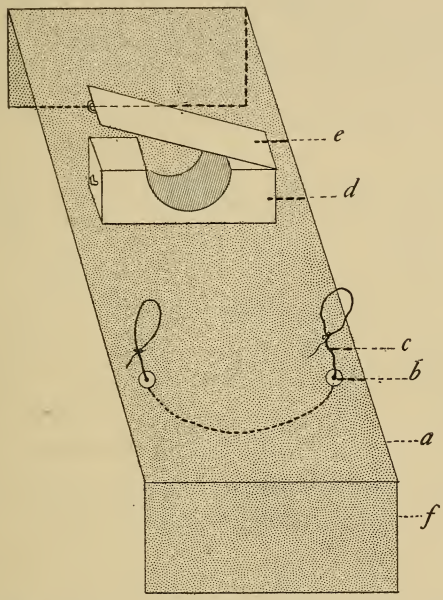

FIG. 51.—BACTERIOLOGICAL SPECIMENS. Guinea-pig holder. *a*, Board 18 inches long by 8 inches wide supported by two ends, *f*, 8 inches wide by 6 inches high; *b*, hole through which leather thong *c* passes. The slip-knots are passed over the hind legs after the wooden strip *e* has been fastened so as to hold the head in the wooden block *d*.

it so as to cause steaming for two to five minutes. Apply fresh stain as often as necessary. The slide may also be heated by placing it across the rings of a boiling water-bath. Wash off the stain by flooding the preparation with water. The Czaplewsky solution is now run over the preparation by dropping from the bottle until the thinner parts of the smear are only a very faint pink. Wash with water and stain with Loeffler's methylene blue for one-half minute. Wash, air dry and examine with oil. The

tubercle bacilli are red while all other parts of the preparation are blue unless there are heavy masses in the smear which may retain the red.

Capsule Stain.—Stain with McJunkin's polychrome stain for protozoa after fixation in absolute alcohol-ether.

Flagellum Stain (Löwit).—Fix in 10 per cent. formalin for one hour. Great care must be taken to preserve the flagella in making the preparation. Place the freshly prepared mordant (10 c.c. of 20 per cent. tannic acid, 5 c.c. saturated aqueous solution of copper sulphate and 1 c.c. of saturated alcoholic solution of fuchsin

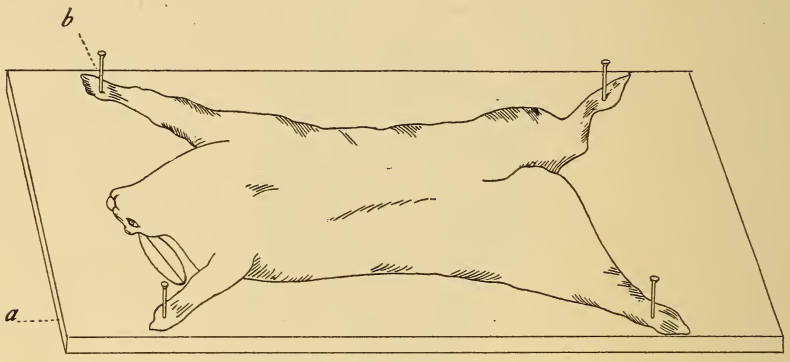

FIG. 52.—BACTERIOLOGICAL SPECIMENS. Autopsy board. *a*, ¾-inch board of soft wood large enough for rabbit; *b*, small wire nails.

(neutral); filter) on the preparation for three minutes; wash and stain five minutes with the Gram's staining solution steaming gently. Wash, air dry and mount in balsam.

Spore Stain.—To establish the presence of spores with certainty it is necessary to make a differential stain. (1) Young spores: Fix in flame. (2) Five per cent. chromic acid for ten minutes. Wash. (3) Carbol-fuchsin, steaming gently for ten minutes. Wash. Examine in water. The spores must be red. (4) Differentiate in 95 per cent. alcohol until only the spores remain red. Wash and examine in water. (5) Stain with Loeffler's methylene blue. Dry and mount in balsam.

Special Specimens.—Some bacteriological specimens sent to the laboratory require a special technic in order to determine the

organisms present. Specimens of urine from catheterized ureters are frequently requested to be used for guinea-pig inoculation. In cases where the gas bacillus or tetanus is suspected, a special procedure is required. The request for such special examinations must always be written on the brown slip by the clinician.

CEREBRO-SPINAL FLUID.—Cloudiness, if no blood is present, suggests meningitis. If fresh make a preparation in the blood-counting chamber with the undiluted fluid. The number of cells in 1 c.c. should not exceed twenty.

Centrifuge in a 15-c.c. sterile centrifuge tube, remove the sediment from the bottom with a sterile capillary pipette and plant the sediment on blood serum, ascitic agar and blood agar; incubate and examine the cultures daily. Also make a smear from the sediment and stain with polychrome stain for blood for a differential count of the cells.

Estimate the globulin in the clear supernatant fluid by adding 0.5 c.c. of 10 per cent. butyric acid to 0.2 c.c. of the cerebro-spinal fluid in a small test-tube. Heat and add 0.1 c.c. of normal sodium hydrate. A precipitate indicates globulin (Noguchi).

If a Wassermann is done use 1 c.c. of the fluid, instead of 0.1 c.c. which is the usual amount of the patient's blood-serum used for this test.

FLUID FOR TUBERCLE BACILLI.—Centrifuge in a sterile centrifuge tube. Decant supernatant fluid. Stain sediment by the Ziehl-Neelsen method. If there are no tubercle bacilli and few streptococci or none at all, a guinea-pig may be injected (Fig. 47) on the request of the clinician.

Inject ½ c.c. of the sediment remaining in the bottom of the centrifuge tube subcutaneously in the right groin of a 250-gm. guinea-pig and ½ c.c. intraperi-

FIG. 53.—BACTERIOLOGICAL SPECIMENS. Fermentation tube. Place the 55 by 7-mm. test-tube *a* upside down in the bouillon in the 155 by 16-mm. test-tube *b* before sterilization. The air is driven out of *a* during the sterilization.

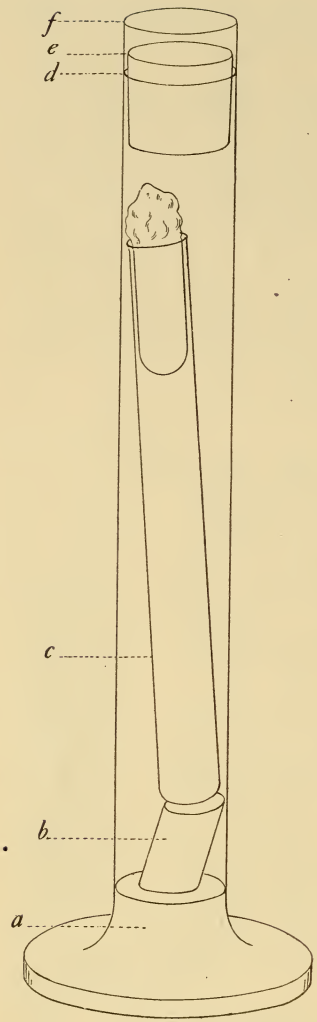

FIG. 54.—BACTERIOLOGICAL SPECIMENS. Anaerobic culture cylinder. *a*, 100-c.c. graduate; *b*, piece of cork to support culture tube; *c*, culture tube; *d*, upper end of graduate; *e*, tightly fitting stopper; *f*, paper wrapped about the upper end of the cylinder into which the paraffin is poured.

toneally. Autopsy (Fig. 52) at the end of six weeks. Examine the tissue of the right groin, the spleen, liver, peritoneal surfaces and the lungs for tubercles by making smears on slides and staining for tubercle bacilli and by making colloidin sections.

PUS OR CURETTINGS FOR B. AEROGENES CAPSULATUS.—Smears show a Gram positive bacillus about the size of the anthrax. Capsules are often demonstrable; the bacilli rarely form long chains. If the material is plentiful inject 0.5 c.c. into the ear vein of a rabbit, kill the animal after five minutes and place in the incubator for twelve hours. If the gas bacillus is present, the organs and cavities will contain large amounts of gas.

If only a swab has been sent for diagnosis, inoculate two tubes of litmus milk and two of glucose agar. Place the litmus milk cultures under anaerobic conditions. After twelve hours inject 0.5 c.c. of the litmus milk into the ear vein of the rabbit; kill and incubate as above.

MATERIAL FOR TETANUS.—Examine smears for the Gram positive pin-shaped bacilli. Inoculate a dextrose bouillon fermentation test-tube (Fig. 53) and make a dextrose agar stab. Make the cultures anaerobic by placing the tube in a 100-c.c. graduate (Fig. 54) or other cylinder of heavy glass in the bottom of which there is 1 gm. (estimated) of pyrogallic acid. Add 10

c.c. of 10 per cent. sodium or potassium hydroxide and stratify a layer of paraffin above and around the cork by holding a cylinder of paper snugly about the cork and upper end of cylinder and running the melted paraffin into it. Examine daily. If plates or a number of tube cultures are desired, use the Novy anaerobic jars.

Milk.—To sterile, wide-mouth bottles containing 99.5 c.c. of sterile water, 0.5 c.c. of the milk collected in sterile tubes is added.

The milk is placed in the test-tubes by pieces of sterile glass tubing (3 feet long) kept sterile by wrapping in sterile muslin. These long pieces of tubing are used for stirring the milk in the can before the sample is placed in the sterile test-tubes in which the samples are taken to the laboratory.

One cubic centimeter of this 1–200 dilution is added to one tube of melted agar cooled to 45°C. After mixing pour into a Petri

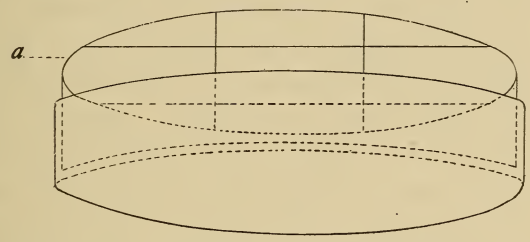

FIG. 55.—BACTERIOLOGICAL SPECIMENS. Petri dish ruled for counting colonies. *a*, Bottom of inverted Petri dish ruled off with a wax pencil into 9 parts.

dish and incubate for forty-eight hours; 0.2 c.c. of the diluted milk is used for making a second plate. Count the colonies after forty-eight hours of incubation. Report the number of colonies per cubic centimeter of milk. Examine small translucent colonies for streptococci. In counting the colonies, rule the bottom of the plate into 9 parts with a wax pencil (Fig. 55).

NOSE AND THROAT CULTURES FOR DIPHTHERIA BACILLI.—With a swab (Fig. 40) rub the suspicious portion of the mucous membrane and with the swab inoculate a tube of Loeffler's blood serum. Incubate over night and stain the eighteen-hour growth with Loeffler's methylene blue. Where a large number of cultures are examined daily, one may make eight preparations on a slide. A pipette with a double curve (Fig. 56) is very convenient for placing the

8 small drops of water on the slide to which the bacteria from the surface of the tube are added by means of the usual platinum loop.

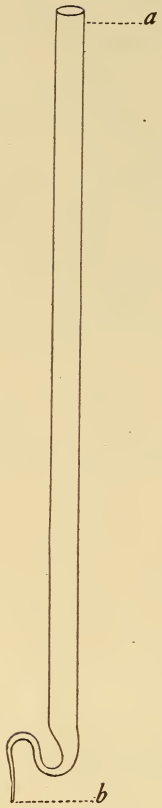

FIG. 56.—BACTERIOLOGICAL SPECIMENS. Pipette for placing minute drops of water on a slide. Made by heating a piece of glass tubing with a 3-mm. lumen in a flame, drawing the heated portion out and curving it as indicated. One or 2 c.c. of water is placed in the pipette from the open end *a*. *b* is touched to the slide at the points at which the small drops are desired.

When the bacilli persist in the nose or throat for more than six weeks a test of the virulence of the organisms may be desirable. To do this make a swab from a culture that shows numerous bacilli of typical morphology and thoroughly wash the swab off in the water of condensation at the bottom of the tube of blood serum. Tubes that are the same length, but twice the diameter of an ordinary test-tube, are the best for this purpose. Repeat the process with the same swab on tubes numbers 2, 3, 4, 5, and 6, passing from one tube to another with the same swab. Flood the surface of the media of each tube after making the dilution and then incubate in the upright position over night. Examine for diphtheria bacilli a number of translucent colonies from the surface of the slants that show well-isolated colonies and inoculate five separate tubes of blood serum from that many positive colonies. The diphtheria colonies are determined by making smears on slides (eight on a slide) and staining with Loeffler's methylene blue.

After twenty-four hours inoculate each of these tubes containing pure cultures into a 350-c.c. Erlenmeyer flask containing 50 c.c. of bouillon. After forty-eight hours' incubation, inoculate a small guinea-pig subcutaneously with 0.5 per cent. of its weight of the forty-eight-hour flask culture.

If the guinea-pig dies within five days go back to the original five tubes containing the five pure cultures and inoculate another flask of bouillon with these. Incubate forty-eight hours and in-

ject a second guinea-pig with 0.5 per cent. of its weight but along with the toxin, inject 0.5 c.c. of antitoxin. If the pig lives five days the bacilli are diphtheria bacilli.

Bacterial Vaccines.—To prepare a vaccine, open up the lesion freshly and make a swab (Fig. 40) with which an agar culture is made. If the culture is contaminated with organisms that are not desired in the vaccine, make a swab from the surface of the mixed agar culture and with this swab make dilutions on the surface of five agar slants as was done on ten blood-serum tubes with the diphtheria bacilli. The tubes are placed in the incubator. The following day colonies are examined and agar subcultures made from the desired ones. After incubation the tubes are filled half-full with saline and the cultures rubbed off with a platinum needle. Place the suspension in a single sterile tube and heat for one hour at 60°C. Make a culture to test the sterility of the suspension.

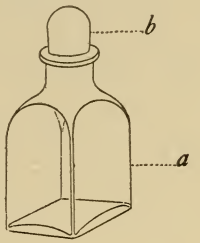

Fig. 57.—Bacteriological Specimens. Vaccine bottle. *a* 10-c.c. bottle; *b*, rubber cap. The bottle and the detached cap are autoclaved. Wipe off the cap with cotton moistened with lysol and insert the sterile needle through it to draw up the desired amount of vaccine. A long 1-c.c. vaccine syringe is used.

To determine the number of bacteria in the suspension, place about 1 c.c. of the sterilized suspension in a watch crystal and fill the blood pipette used for counting the red blood corpuscles to the 0.5 mark with the suspension and then fill the pipette to 101 mark with Hayem's solution to which sufficient acid fuchsin (dry) has been added to give the solution a distinct red color. After the preparation on the slide has stood for one-half hour, count the bacteria the same as the red blood corpuscles are counted.

Suppose there are 1 million in 1 c.mm., then 1 c.c. will contain 1000 million and 0.5 c.c. will contain 500 million, which is an average dose of staphylococcus for example. Dilute the suspension with normal saline so that 1 c.c. contains 1000 million bacteria per cubic centimeter. Add 0.5 per cent. phenol and place in 10 c.c. square sterile bottles and cover with a rubber cap (Fig. 57).

Before using, this rubber cap is wiped off with lysol and the

needle of a long 1-c.c. syringe (graduated in tenths) inserted through it.

A case of furunculosis may for example be given 100 million one day, and after three days 200 million and up to 2000 million. Such vaccines are often given twice per week.

SURGICAL SPECIMENS

Introduction.—To properly care for hospital laboratory work, it is necessary to have a room (clinical laboratory) in which urine, blood, gastric contents, feces, sputum and certain body fluids are examined. These examinations are usually carried out more or less under the direct charge of the clinician. It is not usually required to keep a laboratory record of these examinations as they are filed with the clinical histories by the examiner.

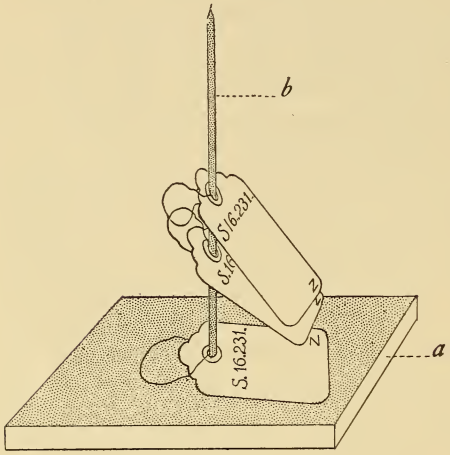

FIG. 58.—SURGICAL SPECIMENS. Surgical tag numbers. *a*, Small block of wood into which a long wire nail *b* has been driven. The tags are merchandise tags (white) measuring 2 by 1¼ inches. The fixation is indicated in the lower right-hand corner. The numbers are consecutive.

In small hospitals it may be necessary to care for the bacteriological specimens and the surgical and autopsy tissue in the same room. In this case the apparatus for the bacteriology and for handling the tissue should be separately placed in different parts of the room, and arranged so that large or small numbers of specimens may be handled in an orderly way.

In large hospital laboratories, aside from the clinical laboratory, there is at least one room for the bacteriological work, one for the tissue work and one for autopsies. The bacteriological specimens are examined and the examinations recorded as indicated under that heading.

A diagnosis on tissue removed during the life of a patient is often desired as soon as it can be made, while the ten days required for the slow paraffin method is no disadvantage with tissue removed at autopsy. For this reason tissue received at the laboratory should be divided into surgical specimens that are removed during life and that receive a surgical number and autopsy specimens that are removed after death and receive an autopsy number. The technic of handling surgical and autopsy tissue is often the same.

Surgical specimens are assigned a number as soon as they reach the laboratory and are disposed of in the ways described below. Tags bearing consecutive numbers are kept on a file for this assignment of numbers (Fig. 58). The numbering is the same as that used for the bacteriological specimens; for example, S 16. 461 is the 461st surgical specimen received in 1916.

Wood Stain.—Desk-tops, table-tops, blocks for staining bottles, and other woodwork should be stainted black. To do this apply with a brush copper sulphate 125 gm., potassium chlorate 125 gm., and water 1000 c.c. and allow to dry. Cover with a second coat, allow to dry and apply aniline hydrochloride 150 gm. and water 1000 c.c. and allow this to dry. Wash with soap and water and after drying, apply linseed oil which should be thoroughly rubbed in. Desks should be cleaned off with a cloth moistened with equal parts linseed oil and chloroform once per week.

Frozen Sections for Immediate Diagnosis.—The sections that may be cut from the fresh tissue and the stains that may be made on these sections give results inferior to those obtained by the colloidin or paraffin method. However, the surgeon may require a diagnosis while the patient is under the anesthetic. In this case tissue is removed and immediately brought to the laboratory by an attendant.

A piece of tissue not larger than 2 or thicker than 1 cm. is cut from the specimen delivered at the laboratory and placed on the disk of the freezing microtome (Fig. 59). The liquid carbon dioxide is allowed to flow into the copper tube (*e*) by turning the valve (*d*)

on the lower end of the cylinder with a long-handled wrench. The carbon dioxide escapes against the disk on releasing the valve (*f*) on the disk end of the copper tube. Let the gas escape until the freezing zone shown by the whitening of the tissue just reaches the top of the block of tissue.

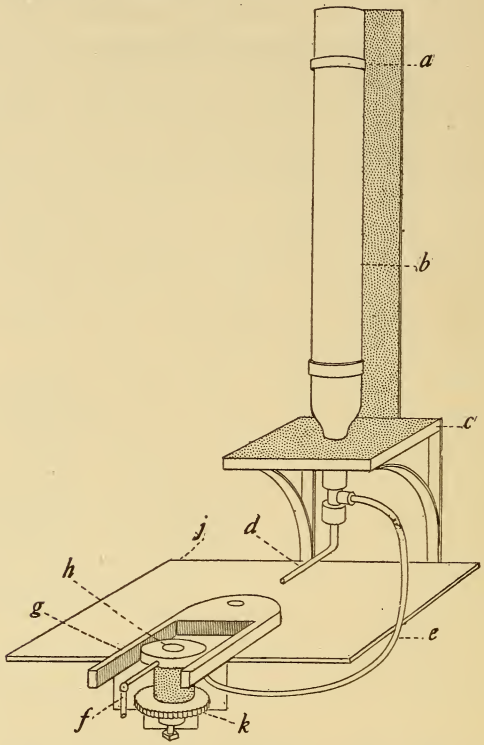

FIG. 59.—SURGICAL SPECIMENS. Freezing microtome. *a*, Chain or leather strap; *b*, CO_2 tank; *c*, 1-inch board with a 4.5-inch hole; *c* is 8 inches above *j* into which the microtome is screwed; *e*, copper pipe with a cock, *f*, on the microtome end (Bausch & Lomb Opt. Co.); *d*, piece of metal pipe attached to the usual short handle wrench that comes with tube; *g*, glassway for knife; *k*, micrometer screw. The cylinder should be placed in the upright position otherwise gas and not liquid flows into *e* as soon as a horizontal cylinder is half empty.

With the knife (Fig. 60) even off the upper surface to the place from which the sections are to be cut. If the tissue is not sufficiently thawed, blow gently on the block and just as soon as thawing begins on the surface rapidly cut a number of sections by turning the milled wheel (*k*) as the knife is rapidly slid back and forth. The sections are removed from the knife blade by dipping the knife into water in a finger bowl.

A slide is placed into this dish and one of the good sections floated onto it and the excess of water removed with a cloth or filter paper.

Stain by dropping 10 drops of polychrome stain for blood onto the preparation from a dropping bottle and after one-half minute diluting with 20 drops of distilled water and staining two minutes. Float the section off by placing the slide in a second dish of water. After washing float the stained section onto a slide, straighten, drop on a square cover at the center of which a small drop of 50 per cent. glycerine has been placed and examine.

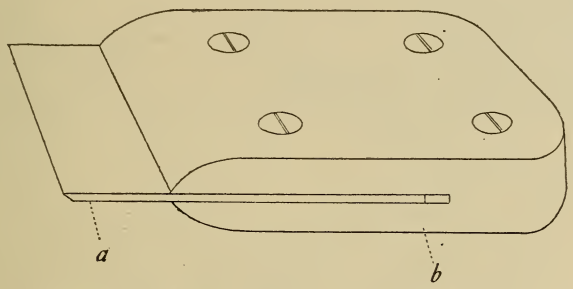

FIG. 60.—SURGICAL SPECIMENS. Knife for cutting frozen sections. 3½-inch planing blade *a*, is set into a wooden handle *b*. The blades when obtained are usually too soft and it is necessary to have them "tempered."

The Spencer freezing microtome (Fig. 61) (No. 880 obtained from the Spencer Lens Co., Buffalo, N. Y.) is widely used for cutting frozen sections and is very satisfactory. The elevation of the specimen by the micrometer screw is automatic.

Not more than five minutes should be taken for the whole procedure. The tissue left after the block has been cut for the frozen sections is treated as indicated under celloidin sections.

Colloidin Sections for Routine Diagnosis—Celloidin sections are used for the routine diagnosis of surgical specimens. By the celloidin method, the sections are stained, examined and reported on the morning following the day the specimens are received. This method enables a technician to do the greater part of the work and large numbers of specimens may be examined without burdensome labor or confusion.

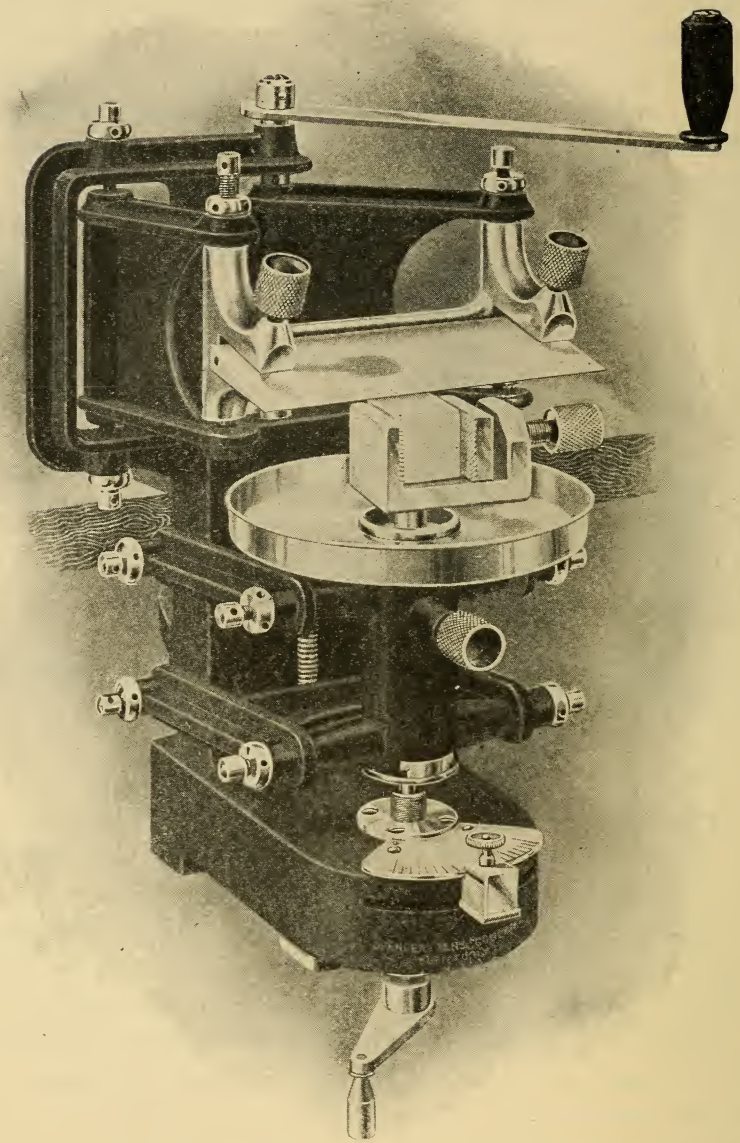

Fig. 61.—The Spencer Freezing Microtome.

Frozen sections of formalin fixed tissue stained with hematoxylin and eosin may be used for routine diagnosis, but it is difficult to get sections of the very small bits of tissue such as curettings without the use of celloidin. The celloidin sections are preferable to sections obtained by a rapid paraffin method.

Care of the Tissue by the Surgeon and its Delivery at the Laboratory.—As soon as the tissue is removed, it is wrapped in sterile gauze and moistened in normal saline; oiled paper (1 foot square) is then wrapped about the gauze (Fig. 63). The brown slip (Fig. 41) is folded and slipped beneath the string. The tissue must be sent to the laboratory as soon as possible. To

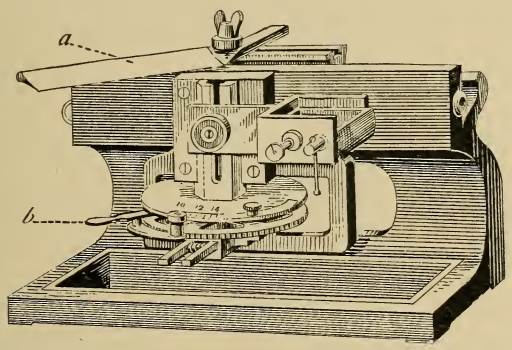

Fig. 62.—Surgical Specimens. Microtome for celloidin sections. Bausch and Lomb microtome No. 3026 with knife No. 3106. Slant the knife *a* so that the entire length of the edge is utilized in cutting off a section, *b*, 12 microns in thickness. 80 per cent. alcohol slowly drops onto the block from bottle (Fig. 71). A number of sections are rapidly cut by drawing the knife back and forth. Transfer the sections from the knife to an Esmarch dish of water with a teasing needle. One of the best sections is picked out and run through the stains (Fig. 72).

secure perfect fixation of tissue, such as is required for making differential stains and for making sections for class work, the tissue must be placed in the fixing solution within five minutes after its removal.

Assignment of a Number to the Specimen.—When received at the laboratory, a specimen is unwrapped and completely disposed of before a second one is unwrapped. The number on the tag at the top of the spindle (Fig. 58) is placed on the bottom of the brown slip and on the sheet (Fig. 64) of a pad which has stamped

on it the number, the fixation and the museum number if a museum specimen is made. The gross description of the specimen is written on this sheet. Then both brown slip and sheet are placed in a clip (Fig. 65) until the diagnosis has been made. The diagnosis is written on the brown slip, when both ar egiven to a technician who makes out the card (Fig. 68) for the laboratory record. The cards are kept in a card index case (Fig. 66). The brown slip with diagnosis written on it is at once returned to the clinician.

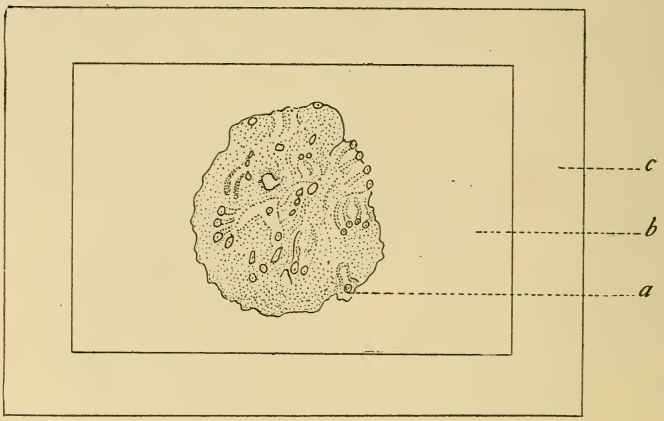

FIG. 63.—SURGICAL SPECIMENS. Surgical specimen wrapped in gauze and oiled paper. *a*, Specimen; *b*, quadruple layer of surgical gauze 8 inches square and moistened with sterile saline; *c*, medium thickness oiled paper 12 inches square. The moist gauze is wrapped about the specimen and the oiled paper is then used to tightly wrap the whole. Tie with ordinary twine and insert brown slip beneath twine.

FIXATION OF THE SPECIMENS.—If a frozen section for immediate diagnosis is not asked for the tissue is unwrapped, examined, and the gross characteristics tabulated. If the specimen is uterine curettings or a bit of other tissue only large enough for a single block, all is placed in the formalin-alcohol bottle (Fig. 69), and the tag from the spindle placed on it. In this case the *Z* on the tag is crossed off and formalin-alcohol substituted. Curettings are held together by placing them with forceps on a piece of filter paper 1 cm. square.

If there is enough of the tissue, pieces are also placed in a

S–16– Z F A M

FIG. 64.—SURGICAL SPECIMENS. Blank sheet for gross descriptions. Tablet paper 5 by 8 inches on which the year number (*e.g.*, S–16–) is stamped in the upper left-hand corner and the three common fixatives in the upper right-hand corner. If the specimen is a museum specimen place number after M.

bottle of Zenker's fluid, unless it is an appendix, leiomyoma, or tube that is known to be of no interest.

The pieces for Zenker fixation are cut after the pieces for diagnosis have been placed in the formalin-alcohol and the Zenker bottle receives the original number tag (Fig. 58). The Zenker-fixed tissue is set aside and put to wash after twenty-four hours. If it appears that a fat stain or a stain for bacteria may be desired, fix also in 10 per cent. formalin.

Technic for the Celloidin Sections.—After the tissue has been placed in the formalin-alcohol (Fig. 66), the further work may

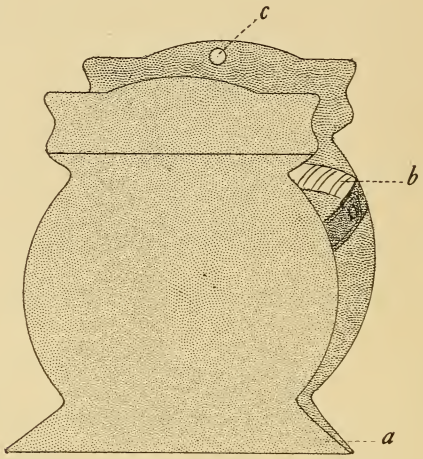

Fig. 65.—Surgical Specimens. Paper clip. *a*, Margin holding paper; *b*, spring; *c*, hole for hanging on a nail. Measures 3 inches.

be placed in charge of a technician. The tissue with tag is moved back with a pair of forceps 10 inches long as indicated in Fig. 69.

After remaining over night in medium celloidin, the blocks of tissue are dipped with long forceps into thick celloidin (the thick is made like medium except that it has 2 ounces of celloidin to the 500 c.c. of alcohol-ether) and placed on a fiber block (Fig. 70) that has the surgical number written with a lead pencil on the back of the block. After a minute in the air the blocks are dropped into a pint Mason jar, half filled with chloroform where they remain for one hour. They are then transferred to 80 per cent. alcohol and celloidin sections are cut with a sliding microtome

(Fig. 57) 12 to 15 microns thick. Eighty per cent. alcohol is dropped onto the block from a large dropping bottle (Fig. 71). The sections are transferred with a teasing needle (Fig. 73) from the knife to a dish of water. The number tag is under this dish.

To make the medium celloidin, remove 1 ounce of celloidin (E. Schering) from the bottle and blot off all water with filter paper. Place in a pint Mason jar and add 500 c.c. equal parts absolute alcohol and ether. Invert the jar for several days so as to dissolve.

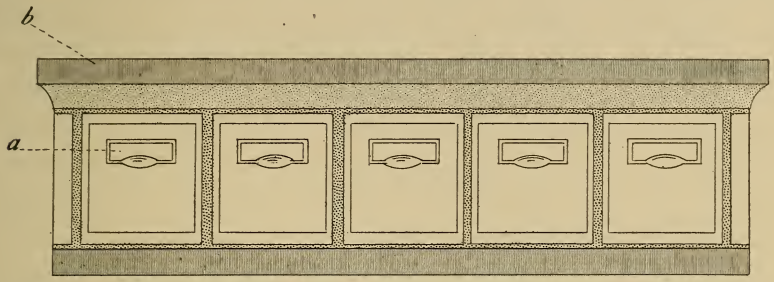

FIG. 66.—SURGICAL SPECIMENS. Card-index case for surgical cards. Section of a card-index case 42 by 16.5 by 8.5 inches. *b*, is top which is removed and replaced as additional sections are added. Shaw-Walker case, Chicago and New York. Five sections will serve for the indexing of the surgical specimens from a large hospital for a number of years and five additional ones will be sufficient for indexing the autopsy specimens. The front card of the drawer, *a*, bears a label, Surgicals S. 15.1 to S. 16.3 for example. From these cards a regional cross index is prepared recording the "P" specimens only. "P" is explained under examination of the celloidin sections.

HEMATOXYLIN EOSINE STAIN FOR CELLOIDIN SECTIONS.—Hematoxylin is most often used as a nuclear stain for celloidin sections as many of the aniline dyes are taken up by celloidin. Esmarch dishes (Fig. 72) are used for the solutions. The procedure is indicated in Fig. 72. After clearing in origanum oil cret. the sections are mounted in colophonium-xylol (500 c.c. xylol to which a pure white colophonium has been added to the desired consistency).

The numbered tag is kept under the dish in which the sections are, being moved forward from dish to dish with the sections. Before the sections are placed on the slide, the slide is numbered (Fig. 74). This eliminates a chance mixing of sections from different cases.

Celloidin sections may be made of tissue fixed in Zenker's fluid. After Zenker fixation the tissue is placed directly into the 95 per cent. alcohol and not in the formalin alcohol. After cutting, the sections of Zenker material are first placed in iodine (iodine 5 gm., and 95 per cent. alcohol 500 c.c.) for fifteen minutes and then in 95 per cent. alcohol until all iodine is removed (one hour or more). The sections are stained in the hematoxylin for thirty minutes instead of five minutes, the time required for the formalin-alcohol tissue. The other technic is the same as for the tissue fixed in the formalin-alcohol.

FIG. 67.—SURGICAL SPECIMENS. Tag for celloidin sections. Merchandise tags 1¼ by 2 inches. The symbol in the lower right-hand corner indicates alcohol-formalin fixation, the usual one used for celloidin sections. The number in the left hand corner indicates that two pieces of tissue are in the bottle. The tag follows the tissue until the section is on the numbered slide.

Tissue fixed in 10 per cent. formalin is treated exactly like the formalin-alcohol fixed tissue except that it is placed directly into the 95 per cent. alcohol instead of the formalin-alcohol.

EXAMINATION OF THE CELLOIDIN SECTIONS.—These celloidin sections are examined and a diagnosis made if possible. In all cases on which a paraffin section of the Zenker-fixed material is desired a "P" is placed on the label. The technician places the bottle of the Zenker-fixed "P"-tissue near the paraffin series (Fig. 78) and a bit of this tissue is cut and started through the series by the pathologist. The Zenker-fixed and the formalin-fixed material of all slides so marked is kept permanently. The Zenker-fixed material as well as any formalin-mixed material of sections not so marked is discarded. The celloidin blocks are kept for one week. The celloidin slides are kept for at least six months. The paraffin slides made from "P"-tissue are kept permanently.

Paraffin Sections.—In those cases in which it is not possible to make a diagnosis from the celloidin section, and in cases where better sections are desired for study, paraffin sections of the

Zenker-fixed material are made. For many of the differential stains, paraffin sections are necessary. In fact celloidin sections are used almost exclusively for diagnostic purposes alone. Paraffin sections of Zenker-fixed material are used for all autopsy specimens.

ZENKER-FLUID FIXATION.—Zenker's fluid (mercuric chloride 500 gm., potassium bichromate 250 gm., sodium sulphate 100 gm. and water 10,000. Add 5 per cent. acetic acid just before using) is the best fixative for general histologic work and the paraffin sections are most often made from tissue fixed in this way. All tissues except the most routine specimens such as appendices, tubes,

S– Diagnosis:

Material: Date:

Name: Service:

Fixation:

History:

Gross:

FIG. 68.—SURGICAL SPECIMENS. Card for surgical records. Card 5 by 7 inches with horizontal ruling. The desired printed headings are indicated.

fibroids, etc., are fixed in Zenker's fluid. Even such tissues as appendices, if obtained within five minutes after removal, should be Zenker-fixed. The original tag (Fig. 58) is placed on the bottle

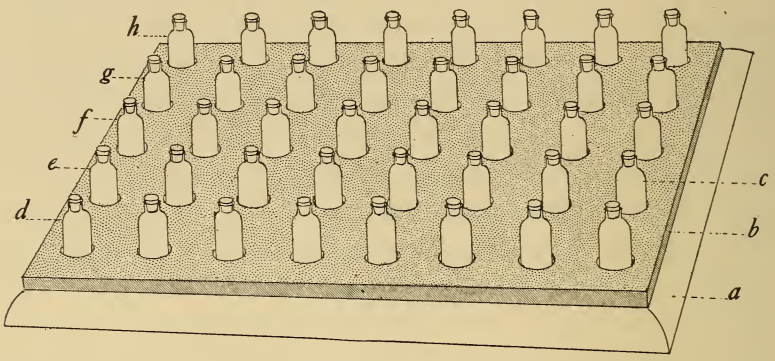

FIG. 69.—SURGICAL SPECIMENS. Block for celloidin imbedding. *a*, ¾-inch board; *b*, ¾-inch board 24 by 14 inches; *c*, cork-stoppered bottles obtained from Whitall, Tatum Co., Philadelphia. The diameter of the bottom of the bottle is 5 cm. and that of the upper part of the mouth is 32 mm. The bottles are 11.5 cm. high. *d*, formalin (1 part)—alcohol 95 per cent. (9 parts) for one to two hours; *e*, 95 per cent. alcohol for one to two hours; *f*, absolute alcohol for one to two hours; *g*, absolute alcohol-ether equal parts two hours; *h*, medium celloidin over night. Eight specimens may be started at one time in this block. The following morning all are ready to be cut by the technician.

containing the Zenker-fixed tissue. Besides surgical specimens, all autopsy specimens are fixed by this method.

For Zenker fixation, the tissue must be cut not more than 4 mm.

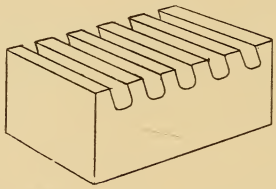

FIG. 70.—SURGICAL SPECIMENS. Fiber block for celloidin sections. Fiber blocks 30 by 20 by 15 mm. may be obtained from Bausch & Lomb Optical Co. The surgical number is written on these blocks with a soft pencil. To clean these blocks remove celloidin by setting aside and letting dry in the air. Remove pencil marks with a moist cloth.

thick, and in the plane in which the paraffin sections are to be cut. An exception is made to this in the case of the brain, heart or other large organ from which a shaving may be cut to show a

superficial inflammatory process. In this case blocks for paraffin sections are cut at right angles to the large surface.

Zenker's fluid is made up in a large 20-liter demijohn in the

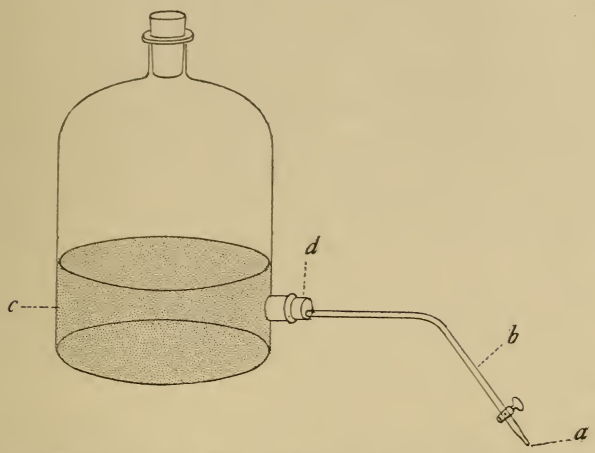

FIG. 71.—SURGICAL SPECIMENS. Alcohol dropping bottle. *a*, 80 per cent. alcohol in a 4-liter bottle provided with a glass tube; *b*, with cock. This tube is connected to the bottle by a rubber cork *d*. The tip of the tube *c* comes directly above the celloidin block. This is done by placing the bottle on a support at the desired height.

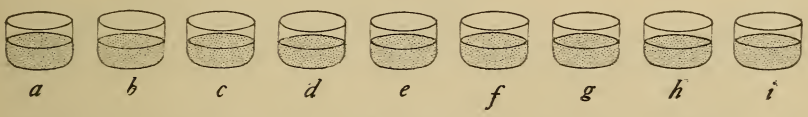

FIG. 72.—SURGICAL SPECIMENS. Esmarch dishes for staining celloidin sections. *a*, Esmarch dish 6 cm. across and 3 cm. deep containing water; *b*, hematoxylin (see Fig. 82) for five minutes; *c*, Czaplewsky until excess of color is removed; *d*, $\frac{1}{500}$ per cent. ammonia for one minute; *e*, water; *f*, $\frac{1}{2}$ per cent. eosin (yellowish, water soluble) for one minute; *g*, 95 per cent. alcohol 1 for one minute; *h*, 95 per cent. alcohol 2 for one minute; *i*, origanum oil cret. for five minutes.

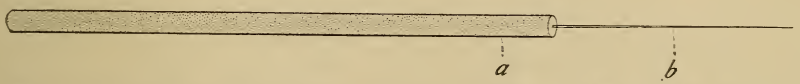

FIG. 73.—SURGICAL SPECIMENS. Teasing needle. *a*, Wooden handle; *b*, steel needle.

bottom of which there is an excess of the bichloride. For convenient use, the Zenker's fluid, 10 per cent. formalin and 80 per cent. alcohol, are kept in bottles (10 liters) provided with cocks (Fig. 76). The acetic acid is added from a burette provided for

the purpose (Fig. 77). The amount of Zenker's fluid should be at least ten times the amount of tissue placed in it.

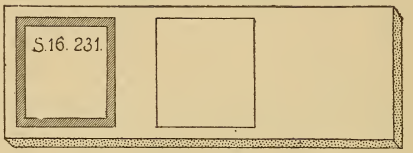

FIG. 74.—SURGICAL SPECIMENS. Slide with surgical number. The slide as it comes from the technician. The cover-glass is 22 mm. square. Before mounting any specimens the labels with numbers are placed on the left end of the slide. Labels are plain with a single narrow black or blue-line border. If for any reason a paraffin section is to be run and the Zenker-fixed material permanently preserved a *P* is placed on the label at the time of examination.

The tissue remains in the Zenker's fluid for twenty-four hours, when it is washed (Fig. 75) for twelve hours or more, and then covered with 80 per cent. alcohol.

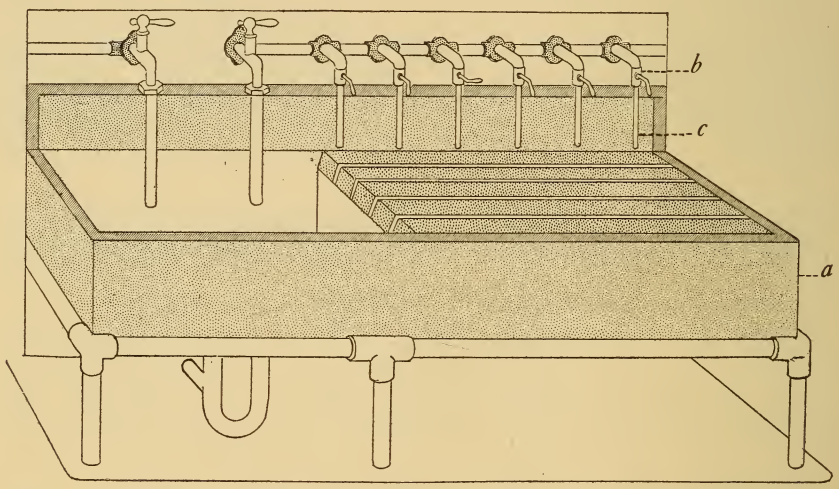

FIG. 75.—SURGICAL SPECIMENS. Sink for washing specimens. *a*, Sink of soapstone. The upper surface inclines and has shallow furrows in it. *b*, water cock which carries a short piece of rubber tubing *c*. This tubing reaches into the bottle during the washing. A small piece of wire gauze may be placed over the tops of the bottles to prevent the escape of bits of tissue.

IMBEDDING IN PARAFFIN.—After remaining over night in 80 per cent. alcohol, blocks not more than 2 to 3 mm. may be cut and started through the paraffin series bottles as indicated in Fig. 78. These blocks, for example from aorta or intestinal wall,

must be cut so that the technician cannot mount them from the paraffin in a way that they will be cut in the wrong direction. That is, cut so that they will be placed in the boats on their flat side without any questioning.

Each morning the specimen with its tag is moved back one bottle. Thus on the seventh day the specimen is removed from the chloroform saturated with paraffin and placed in paraffin (extra white: melting point 52°C., Bausch and Lomb) melted in

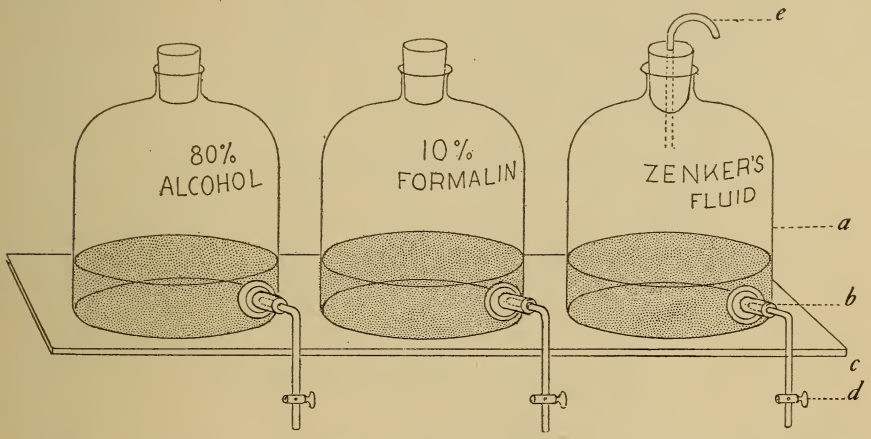

FIG. 76.—SURGICAL SPECIMENS. Zenker's fluid, 10 per cent. formalin and 80 per cent. alcohol bottles. *a*, 10-liter glass bottle with an opening into which a rubber cork is fitted. The rubber cork carries a glass tube with *b* glass cock. A short piece of glass tubing may be placed in the rubber cork and a piece of rubber tubing *c* provided with a pinch cock *d* fitted onto this. *e*, Bent glass tubing to admit air.

paraffin oven (Fig. 79), kept at a temperature below 60°C. The melted paraffin is kept in Esmarch dishes. The number tag is placed beneath the dish that contains the specimen. The specimen is placed in a dish and after about one hour it is transferred to a second dish. The Zenker-fixed specimens remain in the oven about two hours and even three hours as Zenker-fixed material does not shrink in the oven. The tissue is taken from the second Esmarch dish and placed in a paper boat (Fig. 80) made from ordinary paper for this purpose. This boat filled with paraffin which bears the tag number on one end remains in the oven for about fifteen minutes and is then floated on water until the paraffin

completely hardens when the tissue blocks are carefully cut apart. These blocks with the number tags are placed in a pill box of pasteboard (Fig. 81) that bears the number on one end. The blocks are kept permanently in these boxes.

Skin and certain other tissues fixed in Zenker's fluid cut with difficulty if imbedded in the usual way. To correct this it is often

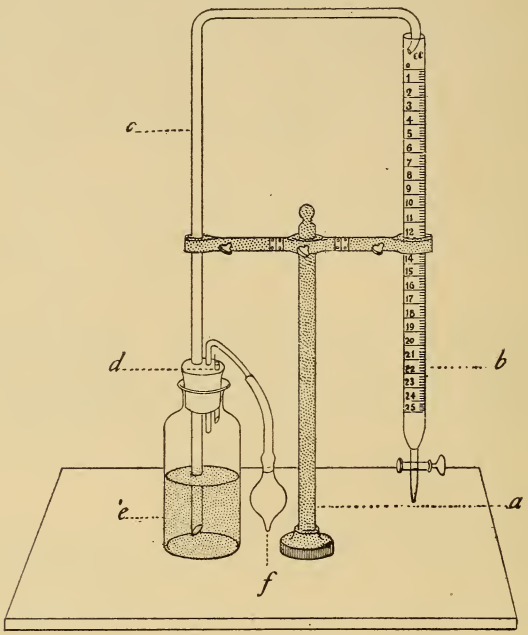

FIG. 77.—SURGICAL SPECIMENS. Burette for glacial acetic acid. *a*, Wooden burette stand; *b*, 25- to 50-c.c. burette; *c*, piece of glass tubing bent as indicated; *d*, third hole in the three-hole rubber cork over which the finger of the left hand is placed while forcing the acid over into the burette; *e*, wide-mouth bottle 6 inches high containing glacial acetic acid; *f*, rubber bulb with a double valve.

advantageous to imbed in paraffin according to the method given under FORMALIN FIXATION.

DECALCIFICATION OF BONE.—Bone must be decalcified. Fix bone sawed into pieces not more than 4 mm. thick in Zenker's fluid, wash and transfer to 5 per cent. nitric for twenty-four hours (forty-eight hours is the maximum). Wash over night. Cut celloidin or paraffin sections.

FORMALIN FIXATION.—Ten per cent. formalin (Fig. 76) (4 per

cent. formaldehyde) is used. Frozen sections are cut from tissue fixed in formalin for the demonstration of fat. Formalin-fixed tissue is also valuable for the staining of bacteria and protozoa present in the tissue. All important specimens are fixed in formalin as well as Zenker's fluid.

For the staining of organisms in the tissue very thin sections must be cut from paraffin blocks. No shrinkage occurs in tissue

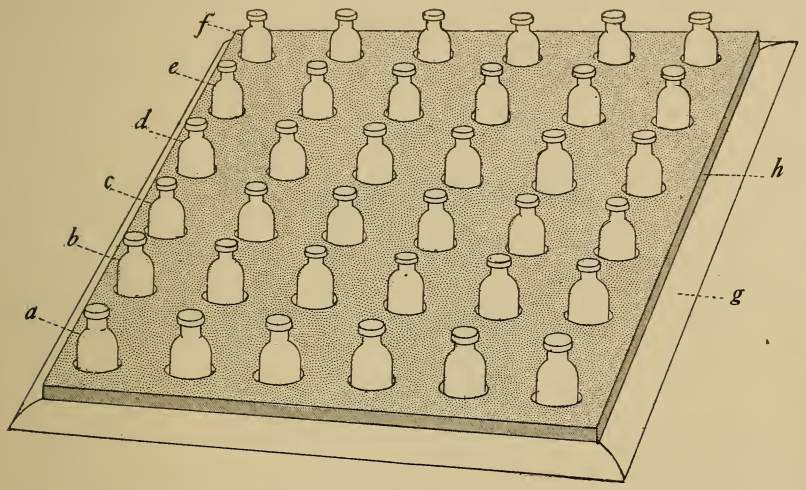

FIG. 78.—SURGICAL SPECIMENS. Block for paraffin imbedding. *a*, 95 per cent. alcohol 1; *b*, 95 per cent. alcohol 2; *c*, absolute alcohol 1; *d*, absolute alcohol 2; *e*, chloroform; *f*, chloroform saturated with paraffin; *g*, ¾-inch board on which is nailed *h*, a ¾-inch board into which the 2⅜-inch holes for the bottles are bored; *g*, is 19 inches square and *h* 18 inches square. The glass-stoppered bottles (Eimer and Amend) are 4⅝ inches high with stopper in place and 2 3/16 inches across the bottom.

during fixation in formalin as happens in the case of Zenker fixation, but during the imbedding process very serious shrinkage takes place unless special care is taken to prevent it.

For this reason formalin-fixed tissue is taken from the formalin and placed in 80 per cent. alcohol for twenty-four hours and then in equal parts of absolute alcohol and cedar oil (cedar oil clearing) for one day when they are transferred to a second bottle of the same. On the third day they are placed in cedar oil, and on the fourth day they are placed in chloroform saturated with 52° paraffin where they remain over night. Transfer to melted 52° paraffin in

the oven for thirty minutes (two changes). The specimens are removed from the oven and blocked the same as Zenker-fixed tissue. The four bottles for this formalin-fixed tissue should be properly labeled and kept in the block used for the Zenker-fixed material (Fig. 78).

Sections from such blocks may be cut and stained with hematoxylin and eosin. The paraffin is removed as indicated under

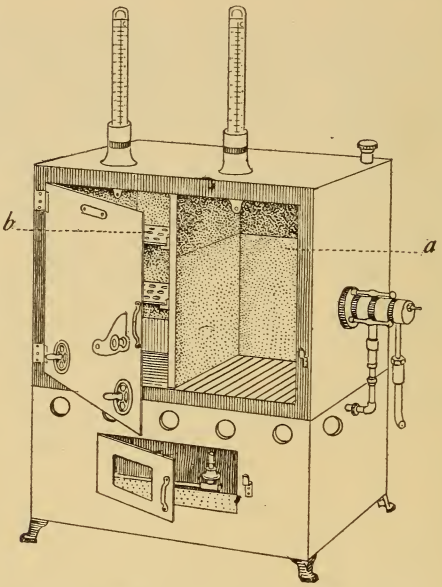

FIG. 79.—SURGICAL SPECIMENS. Paraffin oven. *a*, Compartment in which 52° paraffin (extra white) is kept in a vessel with a pouring snout; *b*, shelves on which Esmarch dishes containing paraffin are kept. Regulate at 55°. This instrument was obtained from Bausch & Lomb Optical Co.

eosin-methylene blue stain. Stain according to the technic given under celloidin sections using a long dish (*b*, Fig. 86) until the 95 per cent. alcohol is reached. Transfer from 95 per cent. alcohol to absolute alcohol and then to xylol rather than to the origanum oil cret. used for celloidin sections. Mount in colophonium-xylol.

ALCOHOL FIXATION.—If 95 per cent. alcohol or absolute alcohol is used, there is a shrinkage of the tissue during fixation, and it may be imbedded in the same way that Zenker-fixed material is imbedded. For the preservation of glycogen so that it may be

stained, pieces of tissue not more than 2 mm. thick are fixed in absolute alcohol.

Cutting Paraffin Sections.—The paraffin blocks whether obtained after Zenker's fluid, formalin or alcohol fixation are cut on a rotary microtome (Fig. 82). For the usual work 7-micron sections (two clicks) are cut but it may at times be desirable to cut from very dense cellular tissue such as liver, 3½-micron sections (one click).

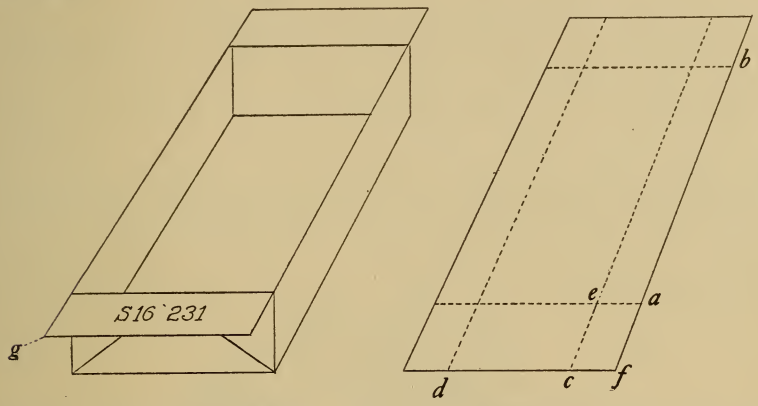

Fig. 80.—Surgical Specimens. Paper box for blocking tissue. Take a rectangular piece of paper 4 by 6 inches for example and fold the ends inward at *a* and *b* and then to two-thirds that distance the sides at *c* and *d*. Unfold and make the line *ae* coincide with *ce* and then bring *f* to the point *c*. Fold back the lip *g* after all the corners have been treated in the same fashion. The number is written on this lip.

As the ribbon comes from the knife it is held with a small pair of fine-pointed forceps, removed from the knife with the forceps and a needle and placed in a dish of water at about 45°C. The ribbon is then separated at the desired points with a heated scalpel. The sections are floated onto clean slides (B. and L. extra white 3 × 1 medium thickness) on which albumin fixative has been placed with a camel's hair brush and the excess wiped off with clean muslin.

To prepare the albumin fixative thoroughly beat up the whites of two eggs with an equal amount of glycerine, add 1 per cent. sodium salicylate and let stand twenty-four hours. Filter through filter paper. The filtrate must be clear. If it is not clear allow to stand several days before filtering.

For routine work it is preferable to use No. 1 cover-glasses 22 mm. by 40 mm. The length of the ribbon placed on the slide should be this length (Fig. 83). If No. 1 cover-glasses 22 mm.

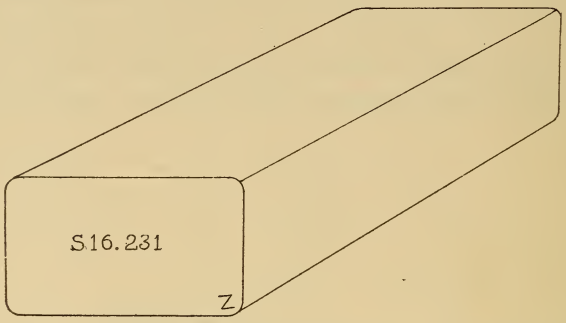

FIG. 81.—SURGICAL SPECIMENS. Pasteboard box for paraffin blocks. White pill box measuring 3⅜ inches by 2¼ inches by 1¼ inches. The number and the fixative are written on one end. These boxes are kept permanently.

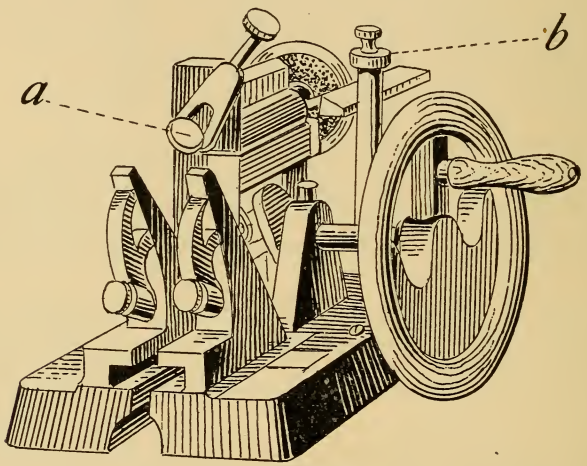

FIG. 82.—SURGICAL SPECIMENS. Rotary microtome. This instrument is obtained from the International Instrument Co., Cambridge, Mass. Obtain at least twelve metal disks *a* to which the paraffin blocks are fastened. *b* bears the numbers by which the thickness of the sections are regulated. When set at 2 for example, the sections are cut two clicks or 7 microns thick. This is the usual thickness for routine work. Obtain at least two Minot microtome knives No. 283 from E. Leitz, New York City.

square are used, the ribbon is placed at the center of the slide and usually consists of a single section from the block.

To fix the specimens to the slides, the slides after the sections

have been placed on them are let dry for a short time and then stood on edge in ten-slide staining dishes (Fig. 84) which are placed in the paraffin oven over night. Sections from only one block of tissues are floated in the dish at a time and the desired number of

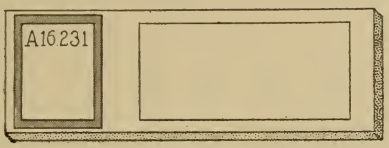

FIG. 83.—AUTOPSY SPECIMENS. Slide with mounted autopsy specimens and label. The slide as it comes from the technician. The cover-glass is No. 1, 22 by 40 mm. The examiner writes the name of the organ below the number and if it shows an interesting lesion one, two or rarely three checks are placed on the label. All autopsy sections are Zenker-fixed and stained with eosin-methylene blue.

sections are placed on slides (Fig. 74) that bear the number of the tissue scratched on the end of the slide by means of a diamond scratch. Whatever stain is to be used, the paraffin is removed with xylol, the xylol removed with alcohol and the alcohol with water.

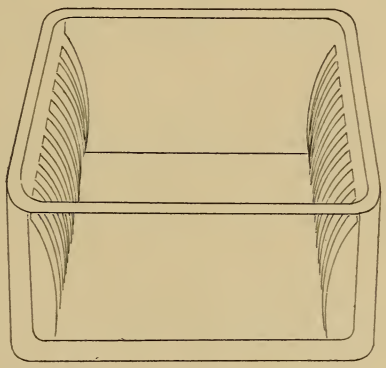

FIG. 84.—SURGICAL SPECIMENS. Ten-slide staining dish. Bausch & Lomb Optical Co.

EOSIN-METHYLENE BLUE STAIN (Mallory).—The slides are removed from the oven and covered with xylol 1 and xylol 2 (Fig. 84) for about fifteen minutes. This removes the paraffin. The absolute alcohol 1 and 2, and the 95 per cent. alcohol 1 and 2 are now poured over the slides one after the other, using a small funnel to pour the solutions back into the bottle. Cover the

sections with water. They are now ready for the stain provided Zenker's fluid has not been used as a fixative.

Mercury must be removed from all Zenker-fixed tissue. The mercury is removed by covering the slides after the second 95 per cent. alcohol with iodine solution (iodine 5 gm. and 95 per

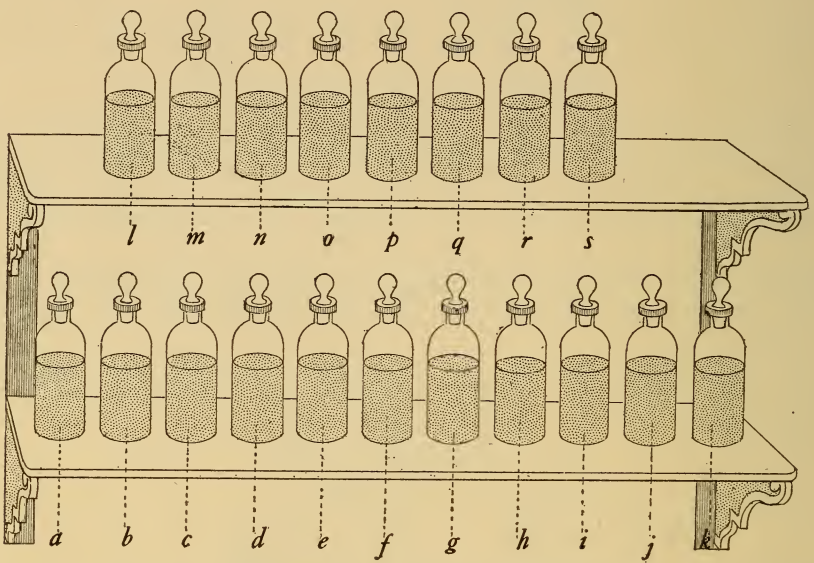

FIG. 85.—SURGICAL SPECIMENS. Bottles for staining solutions. 500-c.c. glass-stoppered bottles 20 cm. high with stopper in place and 8 cm. across bottom are obtained from Eimer and Amend, New York City. *a*, 95 per cent. alcohol 1; *b*, 95 per cent. alcohol 2; *c*, 95 per cent. alcohol for removing iodine; *d*, absolute alcohol 1; *e*, absolute alcohol 2; *f*, xylol 1; *g*, xylol 2; *h*, 25 gm. eosin and 500 c.c. distilled water; *i*, methylene blue 5 gm., potassium carbonate 5 gm. and distilled water 500 c.c.; *j*, phosphotungstic acid (Merck) 5 gm., hematein ammonium (Grübler) 0.25 gm. and water 500 c.c.; *k*, potassium permanganate 1.25 gm. and water 500 c.c.; *l*, oxalic acid 25 gm., water 500 c.c.; *m*, aniline blue (Grübler) 2.5 gm., orange G (Grübler) 10 gm., phosphomolybdic acid (Merck) 5 gm., water 500 c.c.; *n*, acid fuchsin 2 gm., water 500 c.c.; *o*, hematoxylin 5 gm., thymol crystals 1 gm., saturated solution of ammonium alum 500 c.c.; *p*, Czaplewsky (5 c.c. hydrochloric acid sp. gr. 1.19, 5 gm. sodium chloride, 100 c.c. water and 95 per cent. alcohol to 2000 c.c.); *q*, $\frac{1}{500}$ per cent. ammonia; *r*, $\frac{1}{2}$ per cent. eosin (Grübler's yellowish, water soluble); *s*, oil of origanum cretic (Merck).

cent. alcohol 500 c.c.) for fifteen minutes. The iodine must be removed by allowing the slides to remain in the 95 per cent. alcohol until all trace of the brown color has been removed (one to two hours). After removal of the iodine, the slides are covered with water and are ready for the stain.

The eosin-methylene-blue stain is used on Zenker-fixed tissue. The nuclei are usually blue but may be distinctly purplish. The cytoplasm of most cells is pinkish but may stain a blue as is seen in the case of the lymphoblastic cells. Structures such as hyaline in liver cells that are not even visible with other stains are brought out with great distinctness by this stain.

To make the stain, the water is poured from the staining dish (Fig. 84) and the slides covered with a 5 per cent. aqueous solution of Grübler's yellowish, water soluble eosin (Fig. 85) over night. The following morning the eosin is poured back into the bottle, the slides washed in water and then covered with the methylene-blue solution (distilled water 80 c.c. and 20 c.c. of an alkaline methylene blue consisting of methylene blue (Grübler's B.X.) 5 gm., potassium carbonate 5 gm., and water 500 c.c.). The dilution with water should be made just before pouring onto the slides.

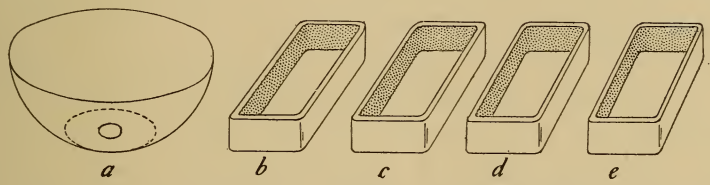

FIG. 86.—SURGICAL SPECIMENS. Dishes for differentiating and dehydrating. Eosin-methylene blue sections. *a*, Finger bowl 4.5 inches across top containing 95 per cent. alcohol to which 1 or 2 drops of colophonium-xylol has been added; *b*, glass dish 3¼ by 1⅛ by ⅝ inches containing absolute alcohol 1. This dish may be obtained from the Marquette Medical School. *c*, absolute alcohol 2; *d*, xylol 1; *e*, xylol 2.

The time that the slides are stained with the methylene blue depends largely on the age of this solution. If it is less than one week old twenty minutes may be sufficient, while if it is one month old forty minutes may be required. The time (average thirty minutes) is best determined by removing a slide from the dish and washing off under the tap. If it is sufficiently stained with the methylene blue, the red does not show macroscopically. After sufficient staining with the methylene blue this stain is washed out of the dish under the tap and the slides allowed to remain in water for a few minutes until each slide can be differentiated.

The differentiation (Fig. 86) of the preparations is important. The slide is placed first in a dish of 95 per cent. alcohol, to which 1 drop of colophonium-xylol has been added and lifted up and down with a needle until a faint pink appears in the preparation, when it is transferred to absolute alcohol in an oblong dish[1] (*b*, Fig. 86). As soon as the first one is differentiated a second slide is placed in the dish and so on until all the dishes (one 95 per cent. alcohol, two dishes absolute alcohol and two dishes xylol) are made to contain a slide. The slides are mounted in colophonium-xylol.

If the slides are too red it is due usually to insufficient staining in the methylene blue rather than to too prolonged differentiation.

Phosphotungstic Acid-hematoxylin Stain (Mallory).—This stain is used on Zenker-fixed material for the demonstration of fibroglia, myoglia and neuroglia fibrils. It is, therefore, especially valuable for the differentiation of cells. The tissue should be fixed five minutes post-operative.

(1) After removal of paraffin, mercury and iodine, place the slide in ¼ per cent. potassium permanganate for twenty minutes. Wash with water. (2) Oxalic acid 5 per cent. for twenty minutes. Wash. (3) Phosphotungstic acid-hematoxylin (hematein-ammonium (Grübler) 0.25 gm., phosphotungstic acid (Merck) 5 gm., distilled water 500 c.c.) over night. This stain is allowed to age for at least one month. Wash. (4) Differentiate in 95 per cent. alcohol one-half to one minute. Pass through absolute alcohol 1 and 2, xylol 1 and 2 and mount in colophonium-xylol.

Aniline Blue Stain (Mallory).—This stain is used on Zenker-fixed material for the demonstration of collagen. It is, therefore, especially valuable for the determination of the presence of fibroblasts.

(1) After removal of the paraffin, mercury, and iodine, wash and place in an acid fuchsin solution (acid fuchsin, (Grübler) 2 gm.,

[1] This dish, measuring at the bottom inside 3¼ by 1⅛ inches and at the top 3⅜ by 1¼ inches, has been made up for the author by the Canton Glass Co., Marion, Ind., and may be obtained in gross lots from them or in small lots from the Marquette Medical School, Milwaukee, Wis.

water 500 c.c.) for twenty minutes. Wash. (2) Aniline blue solution (aniline blue Grübler soluble in water 2.5 gm.), orange G Grübler 10 gm., 1 per cent. aqueous solution of phosphomolybdic acid (Merck) 500 c.c. for twenty minutes. Wash. (3) Differentiate in 95 per cent. alcohol for one-half minute and then pass through the absolute alcohol and xylols to colophonium-xylol.

Elastic Fibril Stain (Verhoeff).—This stain is of value in the morphologic study of tissue, containing many elastic fibrils, such as the walls of blood-vessels. (1) Zenker-fixed tissue; do not treat with iodine. (2) Place for fifteen minutes in the stain. To prepare the stain, dissolve by heat 1 gm. of hematoxylin in 20 c.c. of absolute alcohol in a test-tube and add 8 c.c. of 10 per cent. ferric chloride and 16 c.c. of an iodine solution (iodine 2 gm., potassium iodide 4 gm., and water 100 c.c.). (3) Wash in ½ per cent. ferric chloride until excess of stain is removed (1 minute). (4) 95 per cent. alcohol to remove the iodine (one hour). Wash. (5) Counterstain with ½ per cent. eosin (one-half minute). Wash. (6) Dehydrate in 95 per cent. alcohol (one minute) and origanum oil (five minutes) and mount in colophonium-xylol.

Scharlach R Stain for Fat (Herxheimer).—Place for three minutes, frozen sections cut from formalin-fixed tissue in equal parts of acetone (C. P.) and 80 per cent. alcohol saturated with Scharlach R, transfer to 80 per cent. alcohol for a few seconds, wash in water and counterstain with hematoxylin.

The sections are washed in water and mounted in glycerine jelly. To prepare the jelly, dissolve 50 gm. gelatin in 250 c.c. distilled water and add 250 c.c. of glycerine and 5 c.c. phenol.

The saturated Scharlach R solution should be kept in a 500-c.c. glass-stoppered bottle with an excess of the stain in the bottom of the bottle. To use, filter the saturated solution into an Esmarch dish provided with a cover.

Hemosiderin Reaction.—The iron reaction is valuable where it is desirable to ascertain the nature of a pigment. (1) Frozen, celloidin or paraffin sections of formalin-fixed tissue. (2) Five hours in 2 per cent. potassium ferrocyanide in lithium carmine (dissolve 5 gm. of carmine in 200 c.c. of saturated aqueous solution

of lithium carbonate and add a crystal of thymol). (3) Czaplewsky for seven hours. (4) Wash in running water thirty minutes and pass through 95 per cent. alcohol and origanum oil cret. to colophonium-xylol.

Glycogen Stain (Best).—Rapidly growing tumors and the tissue from cases of diabetes are frequently fixed in absolute alcohol for the demonstration of glycogen. The pieces of tissue not more than 2 mm. thick are carried from the absolute alcohol to alcohol and ether and then to the medium celloidin (see celloidin sections). (1) Stain with hematoxylin and differentiate in the usual way. (2) Place for five minutes in the carmine stain. To prepare the carmine stain, take 6 c.c. of equal parts of methyl alcohol and strong ammonia and add 2 c.c. of the following solution: Add carmine 2 gm., potassium carbonate 1 gm., and potassium chloride 5 gm., to distilled water 60 c.c. and heat to boiling for a few minutes. Cool and add 20 c.c. of strong ammonia. (3) Differentiate for ten seconds in absolute alcohol 80 c.c., methyl alcohol 40 c.c. and distilled water 100 c.c. Then pass through 95 per cent. alcohol (one minute) and origanum oil (five minutes) to colophonium-xylol.

Calcium Reaction.—The calcium of the older deposits in the tissues is chiefly in the form of the carbonate and phosphate. Silver carbonate and phosphate change over to the back oxide in the light.

Frozen, celloidin or paraffin sections of formalin-fixed tissue are used. The sections are placed in 2 per cent. silver nitrate over night, stained with hematoxylin, dehydrated and mounted according to the kind of section. After removing from the silver solution the section, if a frozen one of formalin-fixed tissue, may be stained for fat by the Scharlach R method. In this case it is mounted in glycerine jelly.

Gram-Weigert Stain.—This stain is fairly satisfactory for the demonstration of Gram-positive bacteria in sections. Thin paraffin sections of formalin-fixed tissue are best. (1) Hematoxylin-eosin stain, staining heavier than usual with eosin. (2) Gram's staining solution (see Bacteriological Specimens) five minutes.

(3) Iodine, one minute. (4) Aniline oil for fifteen minutes or more. (5) Xylol 1 and 2 and mount in colophonium-xylol.

McJunkin's Polychrome Stain for Bacteria and Protozoa.—This stain serves for the demonstration of Gram-negative bacteria as well as those staining by the Gram method. It has the additional advantage that the organisms are stained heavily.

One drop of McJunkin's polychrome stain for protozoa is added to the cubic centimeter of distilled water in an oblong staining dish (*b*, Fig. 86) and one end of the inverted slide allowed to rest on the end of the dish (Fig. 84). This is done so as to bring the

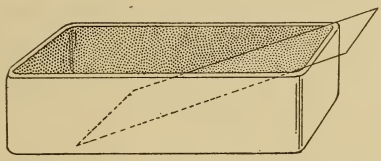

Fig. 87.—Surgical Specimens. Polychrome staining of slides. Dish 3¼ by 1⅛ by ⅝ in. 10 c.c. of stain is placed in this dish and mixed with the lower end of the slide while the other end of the slide is drawn up onto the end of the dish so that the under preparation side is in contact with the stain.

preparation as near to the surface of the stain as possible. The sections formalin-fixed and 3.5 microns thick (one click of the microtome, Fig. 82) are allowed to remain over night in the stain at room temperature or in the incubator. In winter the staining should always be carried out in the incubator. Wash in water and wipe the excess of water from about the preparations with cloth or filter paper. The macroscopic particles of water are removed from the preparations by agitating them in several changes of xylol (pure) containing 5 per cent. absolute alcohol and 1 per cent. colophonium (white). Allow the slides to remain in this solution from three to six hours after all visible water has been removed, when they are passed through two changes of xylol (pure) and mounted in colophonium-xylol. If there is any excess of blue in the sections, this is removed by placing them in the sunlight until the proper differentiation is secured.

Corrosive alcohol may be used for fixation instead of formalin. This consists of a saturated solution of mercuric chloride two parts and absolute alcohol one part.

Stain for Treponema Pallidum in the Tissue (Levaditi).—Formalin-fixed tissue cut into thin pieces not to exceed 2 mm. in thickness is placed in distilled water (two changes) for two days. Transfer to 2 per cent. silver nitrate in a glass-stoppered brown bottle for five days. Wash and place in a bottle containing pyrogallic acid 2 gm., formalin 5 c.c. and distilled water 100 c.c. for two days. Imbed in the usual way in paraffin, cut sections, remove the paraffin with xylol and mount in colophonium-xylol.

Stain for Tubercle Bacilli in the Tissue.—It is often required to stain for tubercle bacilli in the celloidin sections used for diagnosis. A section as thin as can be obtained is floated onto a slide, the excess of water removed with a filter paper and absolute alcohol run onto it from a dropping bottle until completely dehydrated. It is then smoothed out by pressing firmly with filter paper and the slide is placed for five minutes in a 4 by 11-cm. stoppered bottle with 1 cm. of ether in the bottom. Remove and drop 95 per cent. alcohol on the preparation without permitting it to dry and then wash with water. Stain lightly with hematoxylin, wash with water and stain with carbol-fuchsin (see Ziehl-Neelsen stain) for three minutes, steaming over a water-bath or gently over a flame. Decolorize with Czaplewsky until the section is only faintly pink (not more than thirty seconds) and then place in $\frac{1}{500}$ per cent. ammonia for a few minutes. Dehydrate rapidly in 95 per cent. alcohol (thirty seconds) and then in origanum oil (five minutes). Mount in colophonium xylol.

Paraffin sections of formalin, alcohol or Zenker-fixed material are stained in this manner except that the sections are fastened to the slide by placing in the paraffin oven in the usual way. Following this fixation to the slide the paraffin is removed with xylol.

Stain for Leprosy Bacilli in the Tissue (Flexner).—Cut thin paraffin sections of formalin-fixed tissue. (1) Alum hematoxylin for thirty minutes. Decolorize in Czaplewsky and transfer to $\frac{1}{500}$ per cent. ammonia. (2) Carbol fuchsin over night at room temperature. Wash. (3) Iodine (iodine 1 gm., potassium iodide 2 gm., and water 300 c.c.) for one minute. Wash. (4)

Aniline oil two hours or longer until the bacilli can be seen with the oil immersion. (5) Xylol to colophonium-xylol.

Myelin-sheath Stain (Weigert).—This stain colors the fibers that have their myelin sheaths intact. Blocks of tissues for such staining should be not more than 4 mm. thick. They are cut through the gross structures in such a way that in the sections the fibers will be cut transversely. After this stain an area of degenerated nerve fibers appear lightly stained when examined with the low power.

The technic of the stain is as follows: (1) Blocks less than 4 mm. thick from formalin-fixed tissue. (2) Potassium bichromate 5 gm., fluorchrome 2 gm., and distilled water 100 c.c. for four days. (3) Acetate of copper 5 gm., glacial acetic acid 2 c.c., fluorchrome 2 gm., and distilled water 100 c.c. for one day. (4) Imbed and cut 10 micron celloidin sections. (5) Stain the sections for one day in 10 per cent. hematoxylin in absolute alcohol (two weeks old) 10 c.c., saturated solution of lithium carbonate 1 c.c. and water 90 c.c. Wash. (6) Differentiate in borax 2 gm., potassium ferricyanide 2.5 gm., and water 200 c.c. Wash. (7) Dehydrate in 95 per cent. alcohol and origanum oil and mount in colophonium-xylol.

Degenerated Myelin-sheath Stain (Marchi).—In distinction to the Weigert stain, this stain colors only the degenerated sheaths. Place 4-mm. blocks of formalin-fixed tissue in Müller's fluid (potassium bichromate 25 gm., sodium sulphate 10 gm., water 1000 c.c.) for ten days. Transfer to Marchi's fluid (Müller's fluid 200 c.c. and 1 per cent. osmic acid 100 c.c.) for ten days. Wash for one day in distilled water. Cut 10 micron celloidin sections. Dehydrate the sections in alcohol and then pass through chloroform and mount in colophonium-chloroform.

Ganglion Cell Stain (Golgi).—Place bits of the nervous tissue not more than 4 mm. thick in 2 per cent. potassium bichromate for six weeks and then transfer to ½ per cent. bichloride of mercury for six weeks. Dehydrate in absolute alcohol for fifteen minutes and place in medium celloidin for twenty-four hours. Cut thick sections. The sections are dehydrated for one minute in 95 per cent. alcohol, transferred to origanum oil cret. (five minutes) and then to colophonium-xylol.

Chromaffin Cell Stain.—Fix in a saturated solution of potassium bichromate (dissolve 10 gm. potassium bichromate in 100 c.c. water with heat, cool and filter) for twenty-four hours, wash over night in running water, cut paraffin sections and stain with Mallory's eosin-methylene blue stain.

AUTOPSY SPECIMENS

Introduction.—Surgical specimens may be fixed one minute post-operative and are the best for fine histological work. On the other hand, the possibility of examination at autopsy of all the organs has the advantage of showing the relationship and the extent of the disease processes present. An effort should always be made to hold the post-mortem examination as soon after death as possible. If the body is at once placed in a morgue cooled by an efficient refrigerating apparatus, a period of three to six hours between the death of the individual and the autopsy does not affect so seriously the preservation of the tissue. Material from autopsies one hour post-mortem is satisfactory for microscopic examination.

The autopsy room is provided with a zinc, copper or soapstone autopsy table equipped with running water. A board 3 ft. square on which to place instruments and another on which to section organs are provided. Scales with a full set of weights and large scoop pans, a dish with Zenker's fluid and a pint Mason jar containing 10 per cent. formalin are placed on a small table which is drawn up against the foot of the autopsy table. A Bunsen flame, an inch square disk of iron with a handle set in wood for cauterizing, a scalpel to be flame-sterilized, a platinum loop and culture media are also placed on this table. Suitable instruments (Fig. 88) are important. The size and character of these instruments are given in the figure. A printed form-sheet (Fig. 92) is used for taking down the essential data during the autopsy.

The body is placed on its back on the table and a block of wood (Fig. 89) hollowed out so as to support the head is placed beneath the neck. The operator stands at the right side of the subject.

The data, such as the time post-mortem, edema, etc., are determined and recorded as indicated on the slip. A brief clinical history and the probable clinical diagnosis are obtained either from a written record supplied by the clinician or rom the clinician present at the autopsy.

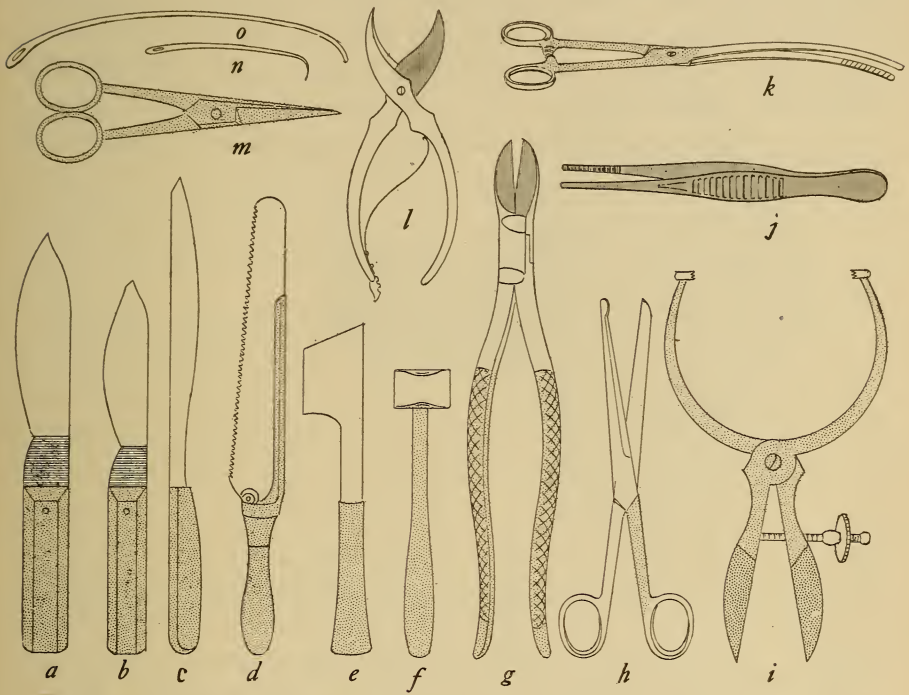

FIG. 88.—AUTOPSY SPECIMENS. Autopsy instruments. Obtained from Codman and Schurtleff, 120 Boylston St., Boston. *a*, Cartilage knife with blade 4 by 11 cm.; *b*, cartilage knife with blade 3 by 9 cm.; *c*, brain knife with blade 2 by 24 cm.; *d*, saw with blade 4 by 25 cm.; *e*, hatchet chisel with a blade 5 by 5 cm.; *f*, metal mallet; *g*, angular bone forceps 45 cm. long; *h*, enterotome 21 cm. long; *i*, head holder, a 12-cm. blade; *j*, mouse-tooth forceps 20 cm. long; *k*, broad ligament clamp with a 12-cm. blade; *l*, costotome 23 cm. long; *m*, scissors 12 cm. long; *n*, curved cutting needle 10 cm. long; *o*, probe 35 cm. long.

External Body Surfaces.—Besides the determination of development, nourishment, edema, etc., already noted, a more careful external examination is made. Positive findings alone are recorded except when there is a special reason to record a negative one.

The external examination should begin with the scalp where

wounds, bruises and scars are looked for. The mouth should be inspected for tumors and inflammatory processes and for abnormal pigmentation.

The neck is examined for enlargements in connection with the salivary glands, thyroid gland and lymph nodes. Asymmetry in the thorax may be important.

The abdomen may be distended with gas (usually within the intestines) or fluid. Large veins may appear superficially in liver cirrhoses. Tumor nodules may appear in the abdominal skin as well as on the skin surface elsewhere. Laparotomy wounds are

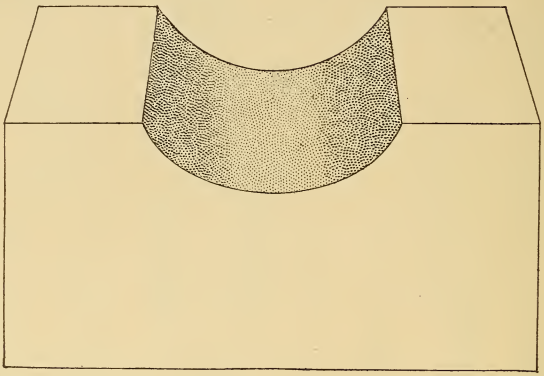

FIG. 89.—AUTOPSY SPECIMENS. Block of wood for supporting wood. Wooden block 12 by 5 by 5 inches with the groove 6 inches across and 3 inches deep.

noted. The external genitals are examined for discharges and old scars. The extremities are examined for abnormalities in size and shape. Tattoo markings on the arms and chest may aid in identification.

Peritoneal Cavity.—An incision (Fig. 90) is made down the median line of the body from a point just above the center of the sternum to the symphysis pubis avoiding the umbilicus. This incision is carried down to the sternum but on the abdomen it extends only through the skin and subcutaneous fat. After completing the main incision, nick into the peritoneal cavity just below the sternum and insert two fingers of the left hand and cut down between these fingers to the symphysis.

Cut the peritoneum and muscles off from the ribs and cartilages

below the thorax and with long sweeps of the cartilage knife strip all the thoracic muscles back with the skin (Fig. 90). Examine the intercostal muscles for trichinella infection. Examine the mammary gland by cutting into it from the muscle surface.

The viscera contained in the peritoneal cavity are examined. Free fluid is looked for especially in the pelvis. The position of the lower border of the liver and of the diaphragm on the two sides is noted. The appendix, the gall-bladder and the general peritoneal surfaces are examined for evidence of inflammation and tumor. Examine the organs contained in the pelvis.

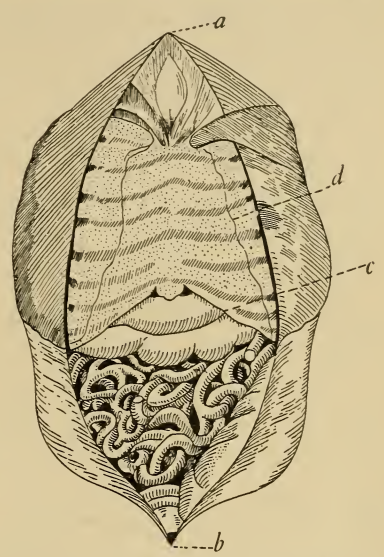

FIG. 90.—AUTOPSY SPECIMENS. Main autopsy incision. Incision extends from an inch above the sternum *a* to the pubis *b*. Strip back the thoracic muscles and cut along the lower border of the ribs at *c*. The ribs are cut along the line *d* with the costotome.

Pleural Cavities.—With the costotome the ribs are cut through from below upward just within the nipple line (Fig. 90), converging toward the costo-chondral junction above until the first rib is reached. The first rib should be cut through not at the costo-chondral junction but outside the mid-clavicular line. This is done by angling the costotome so as to bring the upper blade beneath the clavicle, when it is pushed through between the clavicle and rib and the rib cut.

Now pull the lower end of the sternum upward and with the cartilage knife dissect off the soft tissues from its under surface and cut through the tissues along the lines where the ribs are cut. The sterno-clavicular joint is disarticulated from beneath and the sternum removed. Examine the mediastinal structures for tumor, aneurism and enlarged thymus. The two pleural cavities are inspected; fluid, inflammatory processes, old fibrous adhesions and metastatic tumor nodules are looked for.

Pericardial Cavity.—The position of the apex is noted before the sac is opened. To open the sac catch the pericardium at its center with forceps and first cut upward over the great vessels with scissors. Maintain the hold with the forceps and continue the incision downward along the right side of the heart. From the center of this incision cut downward to the apex.

If death is very slow there may be 100 c.c. of pericardial fluid. If death has not resulted from cardiac paralysis the left ventricle will be contracted. Note adhesions, inflammations and tumor nodules.

Open the pulmonary artery *in situ* by cutting into it with sharp-pointed scissors and opening up toward the lungs. Remove the heart by lifting it upward with the left hand and cutting the vessels from below upward.

Heart.—Weight, 275 gm. Tricuspid 12.5 cm.; pulmonary 9.5 cm.; mitral 10.5 cm.; aortic 7.5 cm.; left ventricular wall 1 cm.

To open the heart, it is placed on a board with the anterior side up. With the enterotome open the right auricle by cutting between the openings of the inferior and superior vena cava. Examine the auricular appendix for thrombi.

Now with one cut of the enterotome open through the tricuspid valve and down the extreme right side of the right ventricle to its apex. From the middle of this incision push the enterotome through the pulmonary orifice and lay it open with one cut.

After examining the mitral orifice from above, push the flat blade through the orifice and open the left ventricle along its left border to its apex. The short blade of the enterotome is inserted into the cavities except in opening the aortic and the pulmonary orifices. From the apex of the left ventricle cut up along the ventricular septum and with one cut lay open the aorta.

Lungs.—Free the lungs by breaking any adhesions that may be present. If the adhesions are very firm, it may be necessary to cut the diaphragm off with the base of a lung. Lift the left lung upward by allowing the root to pull through between the first and second finger and cut through the root from above downward. Right and left lungs are removed in the same way.

Open the vein, artery and bronchus to some distance with probe-pointed scissors. Incise the lung with one long sweeping cut extending from the pleural surface down to the root (Fig. 91), the anterior edge of the lung being held in the left hand with a finger between the lobes.

Spleen.—Weight, 175 gm. Incise the vessels near the hilus and then pull the organ from its position beneath the diaphragm. Examine the capsular surface for infarcts, fibrous thickenings, inflammations, and tumors. Note its consistency. Place on the board and incise in its long diameter and examine the cut surface for the normal lymph nodules and for abnormal collections of cells, such as typhoid nodules. Now make numerous cuts in the short diameter.

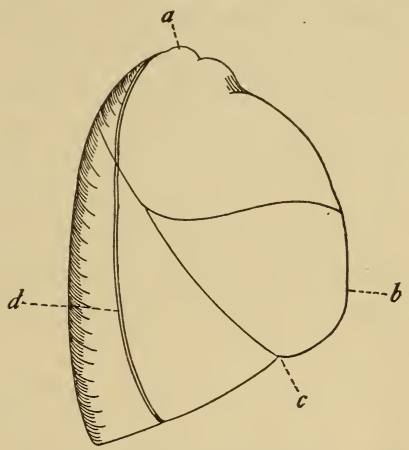

FIG. 91.—AUTOPSY SPECIMENS. Lung incision. Right lung. *a*, Apex; *b*, thin margin; *c*, fissure in which thumb is placed; *d*, long sweeping incision made with brain knife.

Gastro-intestinal Tract.—Cut the omentum off near the transverse colon. Draw the sigmoid colon forcibly upward and with the knife free it from its mesocolon down to the rectum where a double ligature or two clamps are placed on the gut. Cut between the ligatures or the clamps. Free the transverse colon by cutting the lesser omentum connecting it with the stomach. Pull loose the ascending colon and cecum and then by pulling steadily so as to put the mesentery of the small intestine on the stretch cut it off near the gut by a sawing motion of the brain knife until the duodenum is reached. With the scissors cut off the mesentery as near its attachment as possible.

Double clamp the lower part of the duodenum and cut. Separate the stomach from the liver by cutting the hepato-duodenal ligament, noting the contents of the portal vein and hepatic artery and probing the common bile duct. Now cut through the dia-

phragm when the duodenum, stomach and pancreas are removed by dissecting upward beneath these structures. Clamp the esophagus low down and cut.

Remove the intestines to the sink and with the enterotome open the colon along one of its longitudinal muscle bands and the small intestine along the attachment of the mesentery, examining the entire mucous surface carefully. Open the stomach along the lesser curvature and continue the incision through the duodenum using small scissors.

Pancreas.—Weight, 100 gm. Cut the stomach off above and place the pancreas on the board with the duodenum to examine the ducts. Numerous cross-sections are made through the organ. The islets are more numerous in the tail.

Liver.—Weight, 1800 gm. Make a deep incision transversely through both lobes, place the thumb in this incision and pull upward and cut through all attachments, first those of the right lobe and then those of the left.

Examine the capsule for irregularities and the cut surface to determine the condition of the lobules. The markings that correspond to the periportal tissue anastomose, while the areas that correspond to the central portions of the lobules do not. This is important as the color of these structures varies greatly, and in different pathologic processes the same structures appear differently. Make cross-incisions at right angles to the first cut.

Kidneys.—Weight of the two kidneys, 300 gm. Make an incision through the peritoneum and the perinephritic fat along the outer border of the kidney which is then grasped and pulled out of its fatty capsule. Cut the ureter several inches below the pelvis.

Place the kidney in the palm of the left hand and with one sweep of the brain knife cut through to the hilus from the convex border. Strip the fibrous capsule back on either side with forceps. Note the ease with which the capsule strips and the appearance of the kidney surface beneath it. Note the thickness of the cortex at its narrowest point above the pyramids (4 mm.), the appearance of the glomeruli, the straight tubules of the cortex (cortical rays) and the convoluted tubules between these (labyrinth).

Adrenals.—The adrenals are removed with the fat about them and then dissected free from the fat. The right one lies close up against the liver. Incise transversely and note the white center (medulla), the brown zone next this (pigmented reticularis) and the outer yellow part (two outer zones of the cortex).

Genito-urinary Organs.—Strip the peritoneum from the pelvic walls beginning in front of the bladder. Continue to strip down to the lower end of the rectum which is cut through with the knife. Cut through the urethra in front of the prostate in the male and through the vagina in the female and remove the pelvic organs *in toto*.

Open the rectum posteriorly and wash. Open the bladder by cutting up through the urethra and its anterior wall.

Incise the vagina through the bladder wall and continue the incision up through the anterior wall of the uterus and then laterally extend it out through the tubes. Incise the ovaries in the long diameter.

To examine the testicles cut through the tissues beneath the skin on either side of the root of the penis and push the testicles up through the incision. Incise them in the long diameter.

Aorta.—Open the aorta anteriorly along its entire extent and continue the incision down the iliacs.

Neck Organs.—Continue the main incision at least 1 inch above the sternum and if permissible up to the chin. With the smaller cartilage knife dissect up beneath the skin in front and then behind, keeping against the vertebræ. Allow the head to drop back over the block and run the brain knife up in front of the larynx until the point appears in front of the tongue. Turn the knife and with a sawing motion cut back along the ramus of the jaw on either side until the posterior wall of the pharynx is reached which is divided as high as possible. During the entire operation, with the left hand make strong traction on the trachea and esophagus from below. Now pass the left hand up and pull the tongue strongly downward and cut the remaining attachments on either side and remove the organs in one mass.

Take care to cut outside the tonsils.

On the anterior surface, incise each lobe of the thyroid in its longest diameter. Examine the pharynx and incise the tonsils. Now turn the mass over and slit the esophagus down in the median line posteriorly. Slit the larynx and trachea down in the median line posteriorly.

Brain.—Insert a sharp-pointed scalpel at the hair margin just behind the right ear and cutting outward, carry the incision over the vertex of the skull to the same point behind the left ear. Strip back the anterior and posterior flaps by grasping with the hands and cutting only when necessary. Place the hair if long beneath the flaps and wrap a towel about the neck to protect it from blood.

Outline the line to be sawed by cutting through the periosteum and temporal muscle. This line extends from the ends of the scalp incision straight around the forehead over the frontal eminences. Posteriorly two straight lines are carried back to a point just in front of the occipital protuberance.

The head holder is clamped on and screwed down firmly with the clamps in the temporal region. Saw down to and partially through the inner table along the lines marked. Drive in the chisel with the hammer to crack through the inner table. With the chisel inserted in the frontal region pry off the calvarium.

Inspect the dura and then cut through it with scissors along the lines sawed and strip back by cutting the falx cerebri where necessary. Lift up the brain with the left hand and cut the optic nerves, other cranial nerves and the carotids. Draw forward the temporal lobes and cut the tentorium with knife. Cut the cord as low down as possible and remove the brain.

Examine the vessels at the base following the middle cerebrals into the fissures of Sylvius. Place the brain on the board with base down. Spread the two cerebral hemispheres apart and cut through the corpus callosum on each side and extend the incisions into the anterior and posterior cornua, exposing the lateral ventricles and the basal ganglia. Now make two incisions along these cuts with the brain knife carrying them down through the basal ganglia. Cut the corpus callosum in front and reflect back-

ward. By drawing back the velum interpositum the third ventricle is exposed. Outside these two primary longitudinal incisions through the brain substances make two more on each side parallel to the first ones. The cerebellum is cut through in the median line and then two incisions made through each of the halves.

Middle Ear.—With the heavy bone forceps bite off the roof of the middle ear (petrous portion of the temporal bone).

Naso-pharynx (Harke).—Extend the original scalp incision behind the ears downward to the middle of the neck. Dissect the posterior flap down and uncover the upper cervical vertebra. Dissect the anterior flap down and uncover the root of the nose and the upper edge of the orbits. Saw down through the base of the skull in the antero-posterior median line, going slightly to one side through the frontal bone so as to avoid the nasal septum. Cut the ligaments attached about the foramen magnum and forcibly push the two halves of the skull apart. Examine the sphenoidal sinus, the frontal sinus, the nasal passage and the antrum of Highmore.

Bone-marrow.—Make a long incision over the middle of the thigh, dissect and push the muscles aside so as to expose the middle third of the femur. Saw into the marrow cavity of the femur with two incisions separated on the surface of the bone by 3 cm. and in the cavity by 1 cm. With the bone forceps split out this chip of bone, exposing the marrow.

Spinal Cord.—Place the body face down with a block under the chest. Make a median line incision extending the entire length of the spinal column and strip all the soft tissue back on the two sides. Saw through the laminæ and bite off the spinous processes with heavy bone forceps. The arches of the cervical vertebræ are bitten off with the bone forceps. Divide the nerve roots with a scalpel. Remove the cord with dura intact. Slit the dura open posteriorly and anteriorly. Make transverse cuts at a distance of 1 cm.

Fixation of Autopsy Specimens.—As a routine all organs are fixed in Zenker's fluid and in 10 per cent. formalin. The Zenker's fluid (usually 2 liters) is placed in a flat dish on the table. At

AUTOPSY SHEET FOR TEMPORARY RECORD.

Autopsy No. Hours p.m. Age: B.L.
Name: Date: Sex: Color: Develop.:
Nourish.: Rigor mortis: Lividity:
Edema:

Brief clinical history:

Clinical diagnosis:
External body surfaces:
Peritoneal cavity:
Pleural cavities:
Pericardial cavity:
Heart weight:
Lungs weight:
Spleen weight:
Gastro-intestinal:

Pancreas:
Liver weight:
Kidney weight:
Adrenals:
Genito-urinary organs:
Aorta:
Neck organs:
Brain weight:
Middle ear:
Naso-pharynx:
Bone-marrow:
Spinal cord:

Anatomical diagnosis:

FIG. 92.—AUTOPSY SPECIMENS. Autopsy sheet for temporary record. Such sheets should be 8½ by 16 inches.

completion of the autopsy this dish is covered and after twenty-four hours placed to wash. After washing over night the specimens in the dish are covered with 80 per cent. alcohol.

Blocks for paraffin sections are now cut from each organ and placed in a small bottle of 80 per cent. alcohol with tag attached on which is indicated the autopsy number, the number of blocks, and the fixation. The bottle is given to a technician who runs the tissue through the paraffin series (Fig. 78) and cuts sections 7-microns thick which are stained with eosin-methylene blue and mounted in colophonium-xylol.

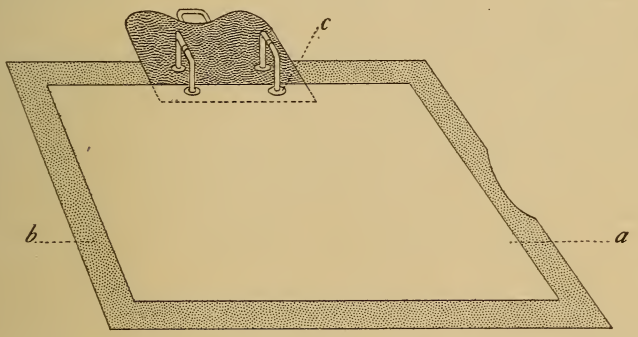

FIG. 93.—AUTOPSY SPECIMENS. Temporary autopsy file. *a*, Pasteboard for protection of autopsy sheets; *b*, board 16½ by 9½ inches; *c*, holes punched in autopsy sheets by a paper punch of the proper width. Obtained from any first-class stationer.

Cover-glasses No. 1,22 × 40 mm. are best for autopsy work, so that several sections may be placed on each slide. The slide bears the autopsy number scratched on it with a diamond scratch. After mounting, plain white labels with narrow black border are placed on the left end of the slides with the autopsy number above.

The slides are placed in a flat tray and the examiner places the name of the organ on each. The sections showing interesting lesions are checked.

The formalin-fixed tissue is used especially for frozen section for a fat stain. The pint Mason jar containing the formalin-fixed tissue is kept for one year and the one containing the Zenker-fixed material in 80 per cent. alcohol is kept permanently.

Cutting the tissue from the organs for fixation in the Zenker's fluid is extremely important. Usually it is best to fix tissue at the time the organ is examined. From the large organs cut blocks of tissue several centimeters in size, place these firmly on the board and with a sharp brain knife cut thin slices of tissue not to exceed 4 mm. in thickness. Select the part to be fixed and in general cut the section in the same direction that the microscopic sections are to be cut. However, in the case of intestine and other thin-walled organs a portion of the wall may be sufficiently thin to fix properly. Include the surface of the organ where possible. Fix an abundance of any organ that shows a macroscopic lesion, especially if the autopsy is fresh.

The autopsies are given consecutive numbers, for example, A 16.101 is the 101st autopsy made in 1916. This method of numbering is explained under surgical specimens. The number and fixation is indicated on a merchandise tag which is attached to the wire clamp of the Mason jar. As soon after the autopsy as possible the operator takes the autopsy sheet (Fig. 92) and from this dictates the autopsy protocol to a stenographer. However, the stenographer may take the dictation during the performance of the autopsy.

The protocol is typewritten on paper measuring $8\frac{1}{2} \times 13$ inches, plain except for one vertical ruling at the left margin. The records for the current year are kept on this file after which they are bound.

The bacteriological findings are added from a bacteriological card that bears the autopsy number. The microscopic findings are dictated for addition to the protocol at which time changes and additions in the anatomic diagnoses are made.

At the time of the autopsy a card is made out with the name of patient, age, sex, service and clinical diagnosis. The number of autopsy is ascertained from these cards. Later the corrected anatomic diagnoses are added to the card.

Museum Preparations (Fig. 94).—It may be required to make museum preparation from either surgical or autopsy specimens. In either case the tissue or organ is given a number (for

example, M 7650 is the 7650th museum specimen made in the laboratory).

If a surgical specimen, brief gross description is written on the blank sheet (Fig. 64) that goes with the brown slip to the technician, and from this the museum card bearing not only the museum number but also the surgical or autopsy number is at once made out.

If an autopsy specimen, a brief gross description is taken from the temporary autopsy sheet and from this the museum card is at once made out. There is a spindle on which muslin slips 1 × 4

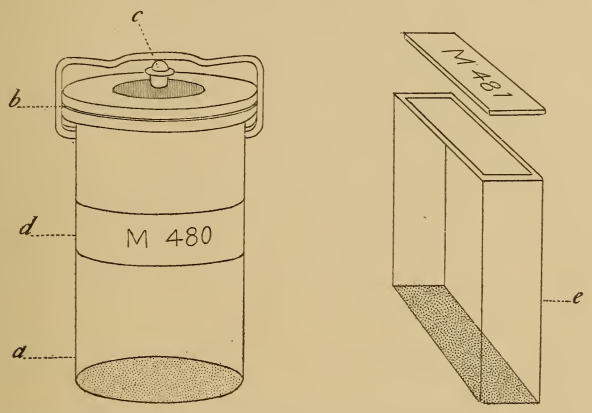

FIG. 94.—AUTOPSY SPECIMENS. Museum preparations. *a*, Round museum jars in which a heart or other specimen that requires removal for demonstration is placed; *b*, rubber band; *c*, screw for tightening the cover; *d*, the museum number consisting of a plain letter and number. The letters and numbers are obtained from The Tablet and Ticket Co., 624 W. Adams St., Chicago. *e*, Rectangular museum jars in which the specimens are sealed. Both round and rectangular jars may be obtained from Eimer and Amend, New York City.

cm. are kept. These bear the consecutive museum numbers so that the number for a new specimen may be ascertained either from this tag file or from the cards. At the time of making the preparation the surgical or autopsy number, as the case may be, is also placed on the muslin slip.

After attaching the numbered muslin slip to the specimen by means of a large cutting needle and coarse thread, the specimen trimmed and supported in such a way as to show best the lesion present, is placed for several days in a large 5- to 10-gallon crock containing ½ per cent. concentrated ammonia in 10 per cent.

formalin. Transfer to 95 per cent. alcohol containing 10 per cent. glycerine until the desired color is secured (about two hours). Place in a third jar containing Russian mineral oil number 2. After a week or more in this jar place in oil in suitable museum jars (Fig. 94) with the museum number placed on top or sides with mucilage letters and numbers. These letters of the size desired may be obtained from the Tablet and Ticket Co., 624 W. Adams St., Chicago, Ill.

The jars are sealed with a combination wax consisting of 300 gm. bee's wax, 100 gm. rosin and 100 gm. soft black rubber tubing melted up in a porcelain dish in which the wax is kept permanently. The wax is melted and applied to the surface to be sealed with a camel's hair brush. A flame from a Bunsen burner may be used to melt any irregularities.

Hand Lotion (Mallory).—In handling museum specimens avoid getting formalin on the hands. If the hands become roughened a hand lotion may be used. This lotion is prepared by placing 1000 c.c. of distilled water, 50 c.c. U. S. P. glycerine, 20 gm. boric acid and 15 gm. (unpowdered) gum tragacanth (the amount varies with the quality) in a bottle and shaking for four days. Then filter through a towel onto which 1 c.c. of oil of geranium has been poured. A good grade of tragacanth should be used.

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